FULL PAPER *Theriogenology*

Controlling Highly Prevalent *Staphylococcus aureus* Mastitis from the Dairy Farm

Hajime NAGAHATA[1)]*, Hiroyasu ITO[1)], Hiroshi MARUTA[1)], Yuzo NISHIKAWA[1)], Hiromi SUSUKINO[1)], Susumu MATSUKI[1)], Hidetoshi HIGUCHI[1)], Takeshi OKUHIRA[2)] and Akira ANRI[3)]

[1)]Department of Animal Health, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069–8501, [2)]Rakuno Gakuen University Farm, Ebetsu, Hokkaido 069–8501 and [3)]Large Animal Clinic and Research Center, Ebetsu, Hokkaido 069–0805, Japan

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ABSTRACT. In 57 Holstein cows where the dairy farm uses a milking parlor system, the somatic cell count (SCC) increased persistently in the bulk milk (monthly mean 52.3×10^4 cells/m*l*; range 21 to 94×10^4 cells/m*l*). We detected *S. aureus* in 24 (41.2%) of the 54 lactating cows and in 29 (12.8%) of 227 quarters of the 57 milking cows in the herd. A control program was implemented in an effort to eradicate *S. aureus* mastitis from this dairy farm. The control plan established improved handling of the lactating cows, improved milking procedures, dry-cow therapy, and culling of infected cows. The program was monitored for 3.5 years by frequent checkups on the rate of *S. aureus* infection, the SCC, and the changes in milk composition. Eighteen months after the control program was started, the rate of *S. aureus* infection in the quarter milk decreased dramatically, and no *S. aureus* isolates were found in the milk of the remaining cows. The SCC in the bulk milk of the herd dropped to a monthly mean of $<20 \times 10^4$ cells/m*l*. In conclusion, the control program was effective for controlling persistent *S. aureus* mastitis in this dairy herd.

KEY WORDS: dairy herd, long-term monitoring, mastitis control program, preventive medicine, *Staphylococcus aureus*.

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Bovine mastitis is a costly disease that affects the dairy industry adversely. *Staphylococcus aureus*, a contagious pathogen that spreads easily from cow to cow, usually manifests as subclinical mastitis with an elevated somatic cell count (SCC) in milk and is associated with decreased quality of the milk [5, 8]. The persistence of *S. aureus* and the poor response of the pathogen to antibiotic therapy make *S. aureus* a common cause of culling. Many attempts have been made to develop effective vaccines against *S. aureus*, but most vaccines do not reduce the incidence of infection [23]. On dairy farms producing high quality milk where other types of mastitis are controlled, *S. aureus* continues to be an important and seemingly ubiquitous mastitis pathogen [14, 20, 22, 24].

Prevention of bovine mastitis and production of high quality milk are strategic to favorable development of the dairy business and proper response to consumer demand [18, 21]. Efforts to control *S. aureus* mastitis in a dairy herd require rigid record keeping of the SCC, of the milk composition, and of the incidence of *S. aureus* in connection with the implementation of rigorous control measures [11].

A Hokkaido dairy farm with increasingly elevated SCC in the bulk tank milk (more than 30×10^4 cells/m*l*) and a high rate of clinical mastitis was referred to our laboratory. On our initial examination of this dairy herd, we collected composite milk samples, and the SCC in those samples was markedly high. From the cultures of the composite milk sample, *S. aureus* was isolated in exceedingly great numbers, thus showing the *S. aureus* infection was highly prevalent in a large proportion of the milking herd. The objective of the present study was twofold: (1) to clarify the contributory causes, i.e., indirect causal factors such as poor hygiene or substandard milking techniques related to the widespread *S. aureus* infection and (2) to control the *S. aureus* mastitis in this dairy herd. Although preventive measures for *S. aureus* infection in dairy herds have long been established, few long-term studies are available on the subject. Our study monitored the herd in Hokkaido for 3.5 years after we instituted a rigorous mastitis control program intended to assure that this dairy farm would be able to produce safe, high quality milk in line with current regulations and consumer demand.

* CORRESPONDENCE TO: NAGAHATA, H., Department of Animal Health, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069–8501, Japan.
e-mail: nagahata@rakuno.ac.jp

MATERIALS AND METHODS

Dairy farm and cows: Dairy farm HK, located in southern Hokkaido, Japan, had 57 lactating Holstein cows at the start of this study. The cows were housed in free-stall barns with concrete floors and divided into 2 milking groups, i.e., lactating and non-lactating. The 57 cows were milked twice a day with a rotary parlor milking system (12 head per platform, New Zealand). Straw and sawdust were used as bedding materials, and the stalls were raked once a day. The milking was performed by the same 2 or 3 dairymen every time.

We visited the dairy farm at least two to three times a month for 3.5 years to collect milk samples and monitor the herd production. Production data for the dairy farm were recorded on a monthly basis for 42 months.

Analysis of bulk tank milk: The bulk tank milk was routinely analyzed for lactose, solid nitrogen fat, and protein at the milk testing laboratory (Dairy Milking Co., Sapporo,

Hokkaido, Japan).

Sampling of milk from quarters: For bacteriological testing, quarter foremilk samples were collected aseptically according to the procedure described by Brown *el al.* [6]. Before sampling, the first 3 to 4 streams were discarded and the distal end of each teat was disinfected with a cotton swab soaked in 70% alcohol. For bacteriological analysis, 2 to 3 milliliters of quarter milk was aseptically collected into 5-m*l*, sterile culture tubes according to the National Mastitis Council [7].

Bacteriological testing: Milk samples (10 μl) collected from the quarters of the lactating cows were swirl streaked onto trypticase soy blood agar plates (Japan Becton Dickinson Co., Tokyo, Japan) containing 5% sheep blood and incubated aerobically for 24 to 48 hr at 37°C. Pathogens grown on the blood agar plates were identified by the protocol described by Brown *et al.* [7]. Quarter milk was considered bacteriologically positive if growth of more than 250 colony forming units (CFU)/m*l* were detected in a sample [13]. *S. aureus* was identified by the hemolytic pattern on the blood agar and by the catalase and positive coagulase reactions [7]. A sample was judged positive for *S. aureus* if the pathogen grew on the blood agar at a rate of less than 250 CFU/m*l*. Other mastitis pathogens were identified on the basis of colony morphology and the hemolytic patterns on the blood agar, Gram's staining, catalase and oxidase testing, and biochemical tests. To differentiate *S. aureus* from coagulase positive *S. hyicus* and *S. intermedius*, the acetoin test (Voges-Proskauer test) was used [7].

Somatic cell count: The SCC in bulk tank milk was evaluated every 2 days by the laboratory of the milking company, and the SCC data were subsequently provided to the dairy farm.

Mastitis control program: The milking protocol we recommended for the dairy herd was the same as that described by Levesque [11]. To determine the contributory causes of such highly prevalent *S. aureus* infection, we checked the milking procedures and implemented the following milking practices: (a) maintenance of the correct milking order, i.e., normal cows first and infected cows or cows showing elevated SCC last; (b) use of disposable plastic gloves for the workers, and individual towels for wiping the teats of each cow; (c) post-milking teat dipping with approved disinfectants; (d) treatment of infected quarters by antibiotic infusion at drying-off; and (e) culling of cows infected with *S. aureus* in 2 or more quarters or cows having chronic *S. aureus* infection.

Statistical analysis: Differences in the infection rates of *S. aureus* in the milk samples were evaluated by Chi-square analysis. Values of $P<0.05$ were regarded as significant.

RESULTS

S. aureus was detected in 24 (41.2%) of the 57 lactating cows and in 29 (12.8%) of 227 quarters of the 57 cows (in one cow, 1 of the quarters was blocked and incapable of giving milk). At the start of our examination, other major mastitis-causing pathogens found in these cows included coagulase-negative staphylococci, *Corynebacterium bovis*, and environmental streptococci (Table 1).

Table 1. Microorganisms isolated from 227 quarter milk samples from 57 lactating cows on the HK dairy farm

Microorganisms	No. of quarters sampled (%)
Staphylococcus aureus	29 (12.8)
Coagulase-negative staphylococci	29 (12.8)
Corynebacterium bovis	38 (16.7)
Environmental Streptococci*	9 (4.0)
Miscellaneous	6 (2.6)
No growth	127 (55.9)
Total	227 (100)

* Streptococci other than *Streptococcus agalactiae*.

In preparation of the udder and teats before milking, warm tap water was sprayed at high pressure to wash the udder and teats as each cow entered the milking parlor. Common towels were used by the dairy workers to wipe the teats. The milking time per cow was not monitored because this rotary parlor system did not have automatic detaching equipment; however, removal of the milking system from the teats tended to require more time than recommended (data not shown). At the onset of the study, we found that the milking order was not being controlled properly and that teat-dipping with 0.1% iodine was not carried out after milking.

In the course of the 3.5-year monitoring period, 24 lactating cows chronically infected with *S. aureus* were culled (Fig. 1). The *S. aureus* infection rate decreased steadily from 42.1% at the time of our first visit to 10.5% seven months after start of the control program. A significant ($P<0.05$) reduction in the positive rate of *S. aureus* in the milk was found one month after the control program was initiated; but the rate of *S. aureus* infection increased to 17–18% at 8 to 10 months, then decreased to 4% at 14 months, and no isolate of *S. aureus* was found in milk samples at 18 months. *S. aureus* infection was found in 2% of the cows at 33 months; however, the infection was ultimately eradicated from this herd.

On our first visit to the farm the mean SCC in the dairy herd was 52.3×10^4 cells/m*l* (range 21 to 94×10^4 cells/m*l*), and on subsequent visits we found that the mean monthly SCC often exceeded the maximum level accepted (with penalty), i.e., 30×10^4 cells/m*l* (Fig. 2). After all the infected cows had been culled, the mean monthly SCC in the bulk tank milk 18 months after the start of the program was 14.5×10^4 cells/m*l* (range 4 to 21×10^4 cells/m*l*), which was well within the approved (without-penalty) level of 20×10^4 or fewer cells/m*l*.

Seasonal variations occurred in the milk fat content and protein content of the bulk tank milk, as shown in Fig. 3.

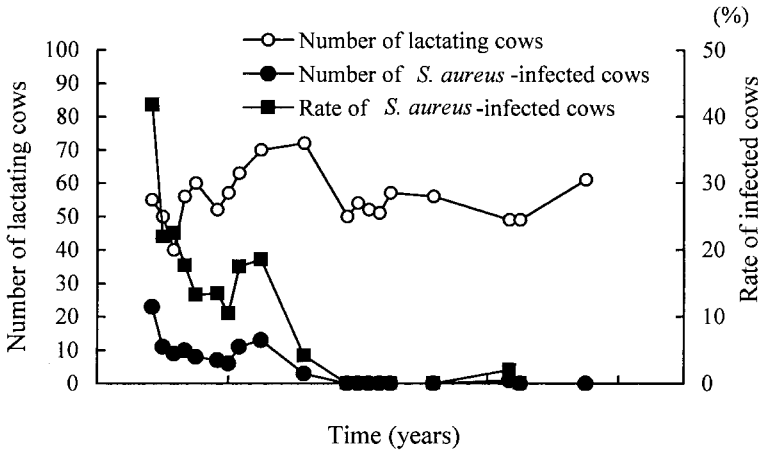

Fig. 1. Fluctuation in the number of lactating cows and cows with *Staphylococcus aureus* infection on the HK dairy farm. Time (years) shows the duration from the start of the mastitis control program to the end of the 3.5-year monitoring period.

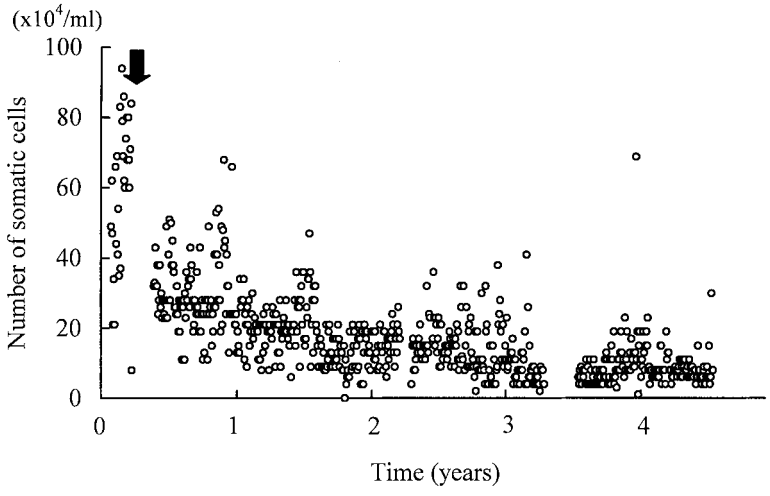

Fig. 2. Fluctuation in the somatic cell count (SCC) of the bulk tank milk at the HK dairy farm. Plot shows data derived from 726 samples of bulk milk collected every 2 days for 3.5 years. Arrows indicate the beginning of the mastitis control program. Time (years) shows the duration after start of the mastitis control program against *Staphylococcus aureus* infection.

DISCUSSION

On the dairy farm in this study, the mastitis control plan we implemented was effective in eradicating *S. aureus* mastitis from the herd. Before the farm was referred to our laboratory, neither the contributory reasons nor the pathogen responsible for the elevated SCC associated with the highly prevalent *S. aureus* infection had been recognized earlier by the dairy producers on this farm, and, consequently, adequate control measures had not been taken before our mastitis control program was implemented. As a result, the dairy farm incurred severe economic losses related to decreased milk production, discarded milk, penalties for SCC elevation in the bulk tank milk, culling of the infected cows, and therapeutic costs [12].

Many factors account for the occurrence of *S. aureus* mastitis on dairy farms [20]. To control these factors, initially we set about to identify the contributory reasons for the high prevalence of *S. aureus* infection at the dairy farm. We observed that critical milking procedures that have proven effective in preventing contagious mastitis were not being carried out on the farm. Hence, we advised the management that their milking techniques ought to be brought up to the standard procedures recommended [6, 11]. The milking time required for each cow was exceeding the normal duration because automatic detaching equipment was

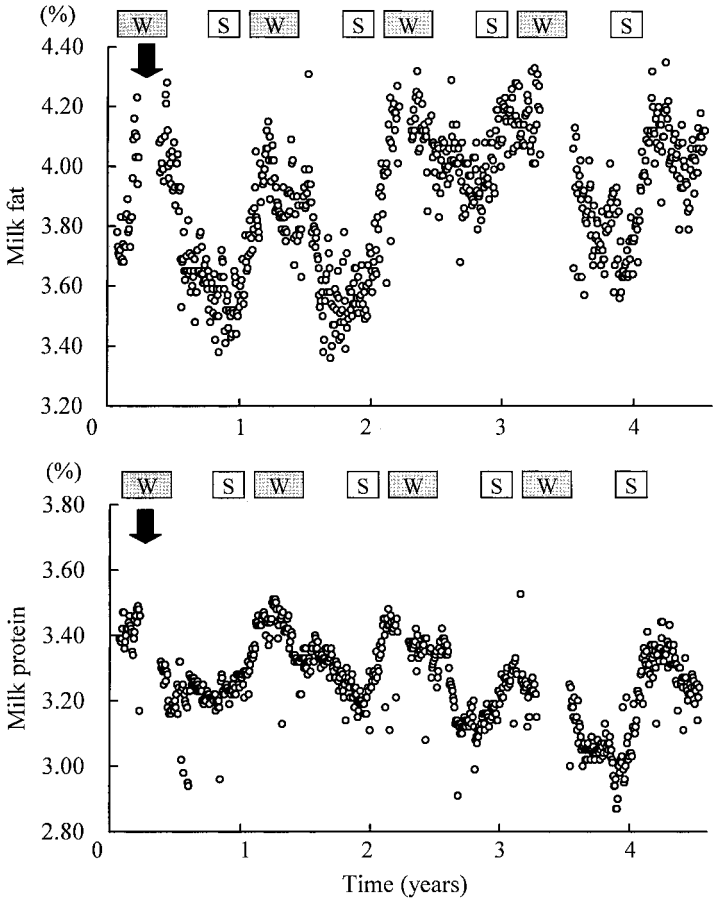

Fig. 3. Seasonal variations in percent of milk fat and protein in the bulk tank milk at the HK dairy farm. Plot shows data derived from 726 samples of bulk milk collected every 2 days for 3.5 years. Arrows indicate the beginning of the mastitis control program. Time (years) shows the duration after start of the mastitis control program against *Staphylococcus aureus* infection. W: winter (December to March); S: summer (July to September).

not part of the rotary milking parlor system used at this farm, and, as a result, the time required to remove the rotary milking system from the teats took longer than is recommended. Presumably, *S. aureus* was transmitted mainly during the milking as the bacteria contacted the teat orifice and surface, both of which can be easily damaged by over-milking. This problem was lessened by adoption of an automatic cutoff system and timely removal of the milking units.

Based on the recommendations for control of contagious mastitis, other preventive measures commonly adopted for control of *S. aureus* mastitis were also introduced [6, 11]. As a result, the average SCC in the bulk tank milk decreased gradually from 52.3×10^4 cells/m*l* to 14.5×10^4 cells/m*l*, as observed 18 months after start of the control program. The moderate increase in *S. aureus* infection detected on the farm 13 to 15 months after start of the control program might have been associated with either *S. aureus*-infected cows incorporated into the lactating herd after parturition or occurrence of a new *S. aureus* infection within the herd. thirty-six months after implementation of the control program, the mean monthly SCC in the bulk tank milk was 6.6×10^4 cells/m*l* and the SCC appeared to be stable at around 10 to 15×10^4 cells/m*l*.

In Hokkaido the positive rate of *S. aureus* in milk samples from cows with clinical mastitis is around 12.8% [1]. In Dutch dairy herds, *S. aureus* is the most common cause of subclinical mastitis in quarters having SCC of more than 25×10^4 cells/m*l*, as reported by Poelarends *et al.* [19]. At the herd level, the prevalence of *S. aureus* in dairy cows is influenced by management and various risk factors [21]. *S. aureus* mastitis occurs sporadically in dairy herds even when the recommended milking protocol, proper handling of dairy cows, and good mastitis control measures have been regularly observed [15]. On the dairy farm in the present study, *S. aureus* infection occurred spontaneously in 2% of the cows 33 months after the infection was eradicated from the herd. In dairy herds where preventive measures are routinely practiced, such widely prevalent *S. aureus* mammary

infection does not occur [11].

Drug therapy for cows infected with *S. aureus* was not the main objective of our study. In therapeutic trials of antibiotics for treating *S. aureus* mastitis, penicillin, novobiocin, Tilmicosin, and Ceftiofur showed little or no efficacy, and the cows with chronic and persistent *S. aureus* were inevitably culled [16, 17]. In our study, we recommended that cows be culled if they had chronic mastitis with 2 or more quarters infected with *S. aureus*. In addition, we recommended dry cow therapy, depending on the condition of the cow. Although the total economic loss due to mastitis could not be calculated, the economic loss associated with the culling of infected cows, decreased production of milk, and cost of treatment would be considerable, and such loss would compare with losses described in the literature [10, 12].

A negative correlation generally exists between the fat content, protein content, and lactose in milk and the number of somatic cells [6]. In our study, the milk fat and protein levels decreased in the summer (July to September), then began to rise again, peaking at around 4.2% and 3.4%, respectively. The seasonal influence appeared to have a greater effect on the compositional content of the milk than did the number of somatic cells or other influences associated with the dairy farm itself. Even in bulk tank milk with a highly elevated SCC due to *S. aureus* infection, seasonal variation in the milk composition continued to be evident. Seasonal changes in milk composition are generally associated with the constituents of feed, temperature, heat stress, environmental changes, and other factors [2–4].

At the HK dairy farm, even after *S. aureus* was brought under control, the SCC rose again in the bulk tank milk of this herd 33 months after start of the mastitis control program. This phenomenon may have been associated with the occurrence of mammary infections caused by environmental pathogens. Our findings and assumptions are consistent with those of other reports [9]. On large-scale dairy farms, maintaining high quality bulk milk requires regular and rigorous monitoring of the SCC and monitoring of the species and number of mastitis-causing pathogens and the incidence of clinical mastitis per month. Safer and higher quality milk production, constructive consultation, and extension services to dairy producers are particularly important in the dairy industry.

In conclusion, the program implemented in this study is effective in controlling *S. aureus* mastitis on the dairy farm. The results of this longitudinal study show that eradication of the pathogen responsible for bovine mastitis depends in large part on the combined efforts to reduce the contributory causes as well. Preventive measures are vastly important for controlling bovine mastitis on dairy farms and for meeting consumer demands. This study bears out that in efforts to prevent bovine mastitis and improve the quality of milk in the bulk tank, paramount concern ought to be directed toward adhering consistently to good dairy practices.

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REFERENCES

1. Anri, A. 1992. Bacteria associated with clinical mastitis in dairy cows in Hokkaido. *J. Vet. Clin.* **351**: 39–43 (in Japanese).
2. Barkema, H. W., Schukken, Y. H., Lam, T.J.G.M., Beiboer, M. L., Benedictus, G. and Brand, A. 1998. Management practices associated with low, medium, and high somatic cell counts in bulk milk. *J. Dairy Sci.* **81**: 1917–1927.
3. Barkema, H. W., Schukken, Y. H., Lam, T.J.G.M., Beiboer, M. L., Benedictus, G. and Brand, A. 1999. Management practices associated with the incidence rate of clinical mastitis. *J. Dairy Sci.* **82**: 1643–1654.
4. Barkema, H. W., Van Der Ploeg, J. D., Schukken, Y. H., Lam, T.J.G.M., Benedictus, G. and Brand, A. 1999. Management style and its association with bulk milk somatic cell count and incidence rate of clinical mastitis. *J. Dairy Sci.* **82**: 1655–1663.
5. Bramley, A. J., Cullor, j. S., Erskine, R. J., Fox, L. K., Harmon, R. J., Hogan, J. S., Nikerson, S. C., Oliver, S. P., Smith, K. L. and Sordillo, L. M. 1996. Methods of controlling mastitis in dairy cows. pp. 39–46. *In*: Current Concepts of Bovine Mastitis. 4th ed. (National Mastitis Council, Inc. ed.), Wisconsin.
6. Brown, R. W., Barnum, D. A., Jasper, D. E., McDonald, J. S. and Schultze, W. D. 1981. Effects of mastitis on milk production, composition, and quality. pp. 4–9. *In*: Microbiological Procedures for Use in the Diagnosis of Bovine Mastitis. 2nd ed. (National Mastitis Council, Inc. ed.), Washington.
7. Brown, R. W., Barnum, D. A., Jasper, D. E., McDonald, J. S. and Schultze, W. D. 1981. Procedures for the identification of specific groups or species of mastitis microorganisms. pp. 10–19. *In*: Microbiological Procedures for Use in the Diagnosis of Bovine Mastitis. 2nd ed. (National Mastitis Council, Inc. ed.), Washington.
8. Fitzpatrick, J. L. 2000. *Staphylococcus aureus* mastitis: past problems, future risks. *Cattle Practice* **3**: 273–277.
9. Gonzalez, R. N., Jasper, D. E., Kronlund, N. C., Farver, T. B., Cullor, J. S., Bushnell, R. B. and Dellinger, J. D. 1990. Clinical mastitis in two California dairy herds participating in contagious mastitis control programs. *J. Dairy Sci.* **73**: 648–660.
10. Groehn, Y. T., Wilson, D. J., Gonzalez, R. N., Hertl, J. A., Schulte, H., Bennett, G. and Schukken, Y. H. 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *J. Dairy Sci.* **87**: 3358–3374.
11. Levesque, P. 2001. The 8-step milking procedure. pp. 19–36. *In*: Profitable Milking. Step by Step to Milk Quality (Piere Levesque. ed.), Institut de technologie agroalimentaire deLa Pocatiere, Quebec.
12. Miles, H., Lesser, W. and Sears, P. 1992. The economic implications of bioengineered mastitis control. *J. Dairy Sci.* **75**:

596–605.
13. Murase, N. 1973. Bovine mastitis. pp. 375–394. *In*: Diagnosis of Infectious Diseases in Domestic Animals(Suppl). (National Institute of Animal Health, ed.), Buneido Publishing Co., Tokyo (in Japanese).
14. Nagase, N., Shimizu, A., Kawano, J., Yamashita, K., Yoshimura, H., Ishimaru, M. and Kojima, A. 2002. Characterization of *Staphylococcus aureus* strains isolated from bovine mastitis in Japan. *J. Vet. Med. Sci.* **64**: 1169–1172.
15. Olde Riekerink, R. G. M., Barkema, H. W., Scholl, D. T., Kelton, D. F. and Keefe, G. P. 2005. Implementation of recommended mastitis prevention management practices and the herd level prevalence of contagious mastitis pathogens on Canadian dairy farms. pp. 587–592. *In*: Mastitis in Dairy Production (Hogeveen, H. ed.), Wageningen Academic Publishers, Wageningen.
16. Owens, W. E. and Nickerson, S. C. 1990. Treatment of *Staphylococcus aureus* mastitis with penicillin and novobiocin: Antibiotic concentrations and bacteriologic status in milk and mammary tissue. *J. Dairy Sci.* **73**: 115–124.
17. Owens, W. E., Nickerson, S. C. and Ray, C. H. 1999. Efficacy of parenterally or intramammarily administered Tilmicosin or Ceftiofur against *Staphylococcus aureu*s mastitis during lactation. *J. Dairy Sci.* **82**: 645–647.
18. Philpot, W. N. 2002. A backward glance - A forward look. pp. 144–155. National Mastitis Council Annual Meeting Proceedings.
19. Poelarends, J. J., Hogeveen, H., Sampimon, O. C. and Sol, J. 2001. Monitoring subclinical mastitis in Dutch dairy herds. Proceedings of the international symposium. pp. 145–149. Mastitis Milk Quality and National Mastitis Council.
20. Roberson, J. R. 1999. The epidemiology of *Staphylococcus aureus* on dairy farms. pp. 38–47. National Mastitis Council. Proceedings.
21. Schukken, Y. H., Barkema, H. W. and Lam, T. J. G. M. 1998. Udder health programs: Present state and future respectives. *Proc. XX World Buiatrics Cong.* **1**: 225–229.
22. Targerson, P. R., Gibbs, H. A. and Anderson, D. B. 1992. High incidence of clinical mastitis due to *Staphylococcus aureus* in two dairy herds with low milk cell counts. *Vet. Rec.* **130**: 54–55.
23. Tenhagen, B. A., Edinger, D., Baumgaertner, B., Kalbe, P., Kluender, G. and Heuwieser, W. 2001. Efficacy of a herd-specific vaccine against *Staphylococcus aureus* to prevent postpartum mastitis in dairy heifers. *J. Vet. Med. A* **48**: 601–607.
24. Waage, S., Mork, T., Roros, A., Aasland, D., Hunshamar, A. and Odegaard, S. A. 1999. Bacteria associated with clinical mastitis in dairy heifers. *J. Dairy Sci.* **82**: 712–719.