Staphylococcus aureus and Streptococcus dysgalactiae in Norwegian herds after introduction of selective dry cow therapy and teat dipping

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The objective was to promote a reduction in the prevalence of *Staphylococcus aureus* and *Streptococcus dysgalactiae* after 2 years of selective dry cow therapies and teat dipping/external teat sealant implementation. Three different dry cow treatments, one long-acting and two short-acting penicillin-based products were tested at herd level together with a negative control teat dipping group, an iodine teat dipping group and DryFlex™, an external teat sealant. The regimes were independently randomly allocated to 178 dairy herds. Yearly bacteriological quarter milk samples were collected from all cows at the beginning of the trial, after 1 year and 2 years. At herd level, a total of 15% of the herds showed no *Staphylococcus aureus* isolates after 2 years, compared with 5% at the beginning. The distribution of *Streptococcus dysgalactiae* infected herds remained the same after 2 years. At cow level, there were no significant differences in the reduction of *Staph. aureus* between the different dry cow therapies and teat dipping regimes. But there was a significant reduction of *Str. dysgalactiae* in the iodine teat dipping group compared with Dryflex™ and the negative control group. The proportional rate of *Staph. aureus* positive quarters was reduced from 65·9% to 54·9% after 2 years. As for *Str. dysgalactiae*, an increase was observed from 14·2% to 15·2%.

Keywords: Herd prevalence, *Staph. aureus*, *Str. dysgalactiae*, teat dipping, selective dry cow therapy.

Total dry cow therapy is one part of a total management system recommended as an important tool in the Five-Point Mastitis Control Plan (Kingwill et al. 1970) to reduce the level of intramammary infections (IMI) during the beginning of the dry period. The use of total dry cow therapy may result in an overuse of antibiotics and selective dry cow therapy programmes have been introduced where the goal has been to optimize treatment of infected cows while minimizing treatment of healthy cows. Nordic countries use selective dry cow therapy as one part of their national mastitis control programmes since it has a well-documented effect (Østerås et al. 1999; Berry & Hillerton, 2002). The chosen selective procedure is scientifically based on trials conducted in Norway and the Netherlands (Østerås et al. 1991; Sol et al. 1997; Østerås et al. 1999; Østerås & Edge, 2000). Since only 2·3% of the *Staphylococcus aureus* isolates, at cow level, are reported to be penicillin resistant, penicillin would be the drug of choice in Norway (Norwegian Cattle Health Service, Annual Report 2004). The overall goal is to treat cows that are expected to respond to treatment and to cull non-responders based on prediction models put forward by Østerås et al. (1999)

An alternative to dry cow therapy is an internal or an external teat sealant used alone or in combination with dry cow therapy. DryFlex™, an external teat seal consists of a physical barrier covering the teat canal. Closing of the teat canal could potentially reduce the amount of pathogens during the dry period and thus reduce the incidence of IMI during the next lactation if the IMI occurs at the time the teat seal is applied.

Teat dipping with a germicidal solution immediately after every milking is another recommended part of a total management system (Kingwill et al. 1970) and is regarded as the single most effective practice for the prevention and control of clinical IMI in lactating dairy cows (Sheldrake & Hoare, 1980; Pankey, 1984; Philpot & Pankey, 1978).

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Table 1. Descriptive information about the 178 participating herds before entering the selective dry cow therapy and teat dipping trial

	n of herd	Mean n of cows per herd (±SD)	Mean BMSCC† cells/mL (±SD)	Median culling rate per cow year (25/75% percentile)	Median incidence of CM‡ per cow-year (25/75% percentile)	n of DCT§ cows in the trial	Previous use of teat dips, all kinds	Previous DCT registered
No dip/Juvanesta	28	20 (±7·9)	118 000 (±43 000)	0·05 (0–0·10)	0·27 (0·16–0·47)	108	3 herds	1 herd
Iodine/Juvanesta	16	18 (±6·1)	120 000 (±37 000)	0·07 (0·04–0·12)	0·35 (0·22–0·45)	50	2 herds	0 herds
DryFlex™/Juvanesta	18	22 (±9·6)	132 000 (±38 000)	0·05 (0–0·11)	0·23 (0·16–0·53)	73	2 herds	1 herd
No dip/Siccalactin	22	24 (±9·6)	103 000 (±37 000)	0·07 (0–0·13)	0·22 (0·14–0·37)	115	5 herds	0 herds
Iodine/Siccalactin	15	23 (±7·7)	117 000 (±21 000)	0·06 (0–0·16)	0·27 (0·15–0·43)	71	8 herds	0 herds
DryFlex™/Siccalactin	23	18 (±5·0)	123 000 (±46 000)	0·08 (0·04–0·25)	0·34 (0·21–0·54)	136	3 herds	1 herd
No dip/Mastipen	21	24 (±9·9)	115 000 (±36 000)	0·06 (0–0·12)	0·35 (0·21–0·50)	74	6 herds	2 herds
Iodine/Mastipen	21	20 (±6·6)	123 000 (±43 000)	0·10 (0·06–0·15)	0·30 (0·21–0·43)	73	7 herds	2 herds
DryFlex™/Mastipen	14	20 (±5·2)	123 000 (±39 000)	0·05 (0–0·08)	0·21 (0·09–0·28)	68	4 herds	2 herds

† Bulk milk somatic cell count presented with SD
‡ Clinical mastitis
§ Dry cow therapy

Research on the efficacy of iodine post-milking teat disinfectants, published since 1980, contains seven trials (National Mastitis Council, 2004) conducted by natural exposure of the bacteria to the teat end (Bray et al. 1983; Eberhart et al. 1983; Nickerson et al. 1986; Oliver et al. 1991; Goldberg et al. 1994). Of the seven natural exposure trials, three trials have a positive control group containing Bovadine 1% iodine solution, which leaves us with four trials with a negative control group. All of these trials assessed a reduction of clinical IMI, but none of them is a large field trial where commercial dairy herds have used teat dipping product during complete lactations.

There are few reports available showing the prevalence of *Staph. aureus* and *Streptococcus dysgalactiae* at herd level. Several studies were conducted at randomized cow or quarter levels within different herds. The problem with such studies is that the herd status is a product of each cow's status. The effect of one therapy group within the same herd could influence the effect of another therapy group as there could be a possible interaction effect between individuals within the same herd. Applying a field trial at herd level avoids this problem. Østerås et al. (2006) conducted a randomized survey throughout Norway in which *Staph. aureus* was isolated from 8·2% of the quarters while *Str. dysgalactiae* was isolated from 1·1% of the quarters. Pitkälä et al. (2004) presented a nationwide mastitis survey in 216 herds in Finland. Among all quarters with bacterial growth, *Staph. aureus* was isolated from 3·4% of the quarters and *Str. dysgalactiae* was isolated from 0·05% of the quarters.

In the Nordic countries, regular teat dipping and dry cow therapy have usually not been recommended. Thus, the fairly high prevalence of *Staph. aureus* and *Str. dysgalactiae* in Norway (Østerås et al. 2006) could be explained by low implementation of dry cow therapy and teat dipping. The objective of the present study was to evaluate the effect of selective dry cow therapy and teat dipping/external teat sealant implemented at herd level measured as a decrease in the prevalence of *Staph. aureus* and *Str. dysgalactiae*.

Materials and Methods

Selection and randomization of the herds

We designed a longitudinal study where the population under study was stratified in six geographical areas in Norway. Our study population originally included 215 commercial Norwegian dairy herds. The first herds were enrolled in October 2002 and the last in May 2003. The descriptive data of the enrolled herds are presented in Table 1. The expected number of cows with *Staph. aureus* was estimated from the herd size together with the percentage of cows with a composite milk somatic cell count (CMSCC) >200 000 cells/ml (cell200) according to an equation generated from regression analyses of routinely mastitis samples in Norway during 1998:

$$(37{\cdot}555 - 0{\cdot}7985 * \text{size} + 0{\cdot}00853 * \text{size} * \text{size} + 0{\cdot}29688 * \text{cell200}) * \text{size} \qquad \text{(Equation 1)}$$

Randomization of selective dry cow therapy and teat dipping regime were conducted as a computerized systematic random assignment. All herds within each geographical area were sorted according to expected number of *Staph. aureus* positive cows (Equation 1). The first herd drew a starting position of A, B or C and was given the dry cow therapy regime A, B, or C accordingly. The next herd was given the next alphabetically ordered regime. The same procedure was followed for randomizing the different teat dipping strategies, but the herds were then sorted according to their size.

The inclusion criteria, at herd level, were: (1) herds had to be members of the Norwegian Dairy Herd Recording System (NDHRS), (2) the farmer was willing to use the selected dry cow therapy and teat dipping regime he/she used in his/her herd, (3) the farmer had to deliver milk to TINE BA (The Norwegian Dairies) during the study period, (4) the farmer must take CMSCC test days monthly during the study period, (5) the farmer had to implement the Nordic recommendations concerning milking routine described by Alfnes & Østerås (1992).

Exclusion criteria were: (1) the farmer withdrew from the study because he/she did not follow the protocol, (2) the farmer withdrew from the study if the local veterinarian suspected that the protocol was not being followed, or if 50% or more of the quarter milk samples were forgotten, (3) herds were withdrawn if co-ownerships were dissolved during the study period.

Selective dry cow therapy and treatment protocols

The protocol and sampling strategy were:

Cows with a geometric mean of the three last CMSCC test-days >100 000 cells/ml prior to drying-off, and cows with a clinical mastitis history during the last lactation were sampled for bacteriological examination before drying-off.

Cows with positive *Staph. aureus* or *Str. dysgalactiae* isolates were treated at drying-off according to the herd's given randomized regimen.

Cows with positive *Staph. aureus* or *Str. dysgalactiae* isolates prior to drying-off and a geometric mean of the three last CMSCC test days >600 000–700 000 cells/ml were assessed for culling after calving, no dry cow treatment were recommended.

The different dry cow therapies were:

A: Juvanesta® Comp. vet. A short acting antibiotic with 300 mg penethamate hydriodide BAN (300 000 i.u. benzylpenicillin) and 300 mg dihydrostreptomycin sulphate (Boehringer Ingelheim Vetmedica AS). If one or two quarters were infected with *Staph. aureus* or *Str. dysgalactiae*, only these will be treated, if three or four quarters had infection, all four will be treated. The treatment was repeated four times with a 24-h interval.

B: Siccalactin® vet. A long-acting antibiotic consisting of 0·17 g Penicillinbenzatin (200 000 i.u.) and 0·4 g dihydrostreptomycin sulphate (Boehringer Ingelheim Vetmedica AS). The intramammary treatment was given as follows: all four quarters were treated once, independent of number of infected quarters.

C: Mastipen® vet. A short-acting antibiotic consisted of 300 mg penicillin (300 000 i.u. procainpenicillin) (VetPharma AS). If one or two quarters were infected with *Staph. aureus* or *Str. dysgalactiae*, only these will be treated, if three or four quarters had infection, all

four will be treated. The treatment was repeated four times with a 24-h interval.

Teat dipping protocol and sampling regime

The different teat dipping regimes were:

A: Negative control group. No teat dipping should be applied in the herd. If any teat dipping or DryFlex™ had been used previously, this practice had to be stopped before the herd entered the study period.

B: Teat dipping (not spraying) with Proactive plus™, containing 0·15% (1500 ppm) iodine (equivalent to 6–8 ppm available free iodine). All teats in all lactating cows were dipped routinely after milking during the whole lactation. At drying-off, cows maintained in tie stalls were dipped 2–3 d post-milking and cows maintained in free stalls were dipped up to the last day of milking. The whole teat was dipped in the iodine suspension and the remaining suspension left in the dip cup was emptied. The dip cup was washed daily after each milking. The iodine teat dipping was provided by DeLaval AS, Tumba, Sweden.

C: DryFlex™, an external teat sealant. All teats in all lactating cows were dipped with DryFlex™ at drying-off, 10 d before expected calving (including heifers) and again if DryFlex™ had fallen off within 3 d of being applied. The teats were washed carefully and disinfected with a DeLaval 70% alcohol pad after last milking. The teats were dried properly before application of DryFlex™. A DryFlex™ application cup was used for each cow. The whole teat was dipped in the suspension. DryFlex™ was provided by DeLaval AS, Tumba, Sweden.

Bacteriological examination of quarter milk samples

Annual quarter milk samples were taken for bacteriological examination at the beginning of the study period, and again after 1 year and 2 years. The two last annual samples were taken as close to 365 d as possible to avoid any seasonal effect as described by Østerås et al. (2006). The first annual samples were collected by the local veterinarians when they taught the farmers the aseptic milk sampling technique. The two last annual samples were then collected by the farmers. The samples were cooled in a refrigerator as soon as possible, at least within a few hours, after sampling and kept cooled until sending by mail as fast as possible to the mastitis laboratory. Samples arriving at the laboratory on Saturday were cooled at the laboratory until processing on Monday, thus avoiding an uncontrolled cooling chain for more than 24 h. Samples were analysed for bacterial growth on blood agar plate (Blood Agar Base, Oxoid Ltd, Basingstoke, UK) mixed with 5% washed bovine erythrocytes. Examination of bacterial growth and diagnostics followed the official Norwegian

procedure (National Veterinary Institute, 1993) and was in agreement with the recommendations of the International Dairy Federation (International Dairy Federation, 1981). Plates were divided into quarters with β-haemolytic *Staph. aureus* streak. Quarter foremilk (0·01 ml) was streaked out on each quarter before incubation at 37 °C±1 °C for 18–24 h. Typical colonies for *Staph.* spp. producing a typical β-haemotoxic zone was classified as *Staph. aureus.* Combined growth of *Staph. aureus* and *Str. dysgalactiae* was classified separately. Other colonies of *Staph.* spp. were classified as coagulase positive or negative using Prolex™ Staph Latex Kit (PRO-LAB Diagnostics, Toronto, Canada). Coagulase positive isolates were diagnosed as *Staph. aureus. Str. dysgalactiae* were classified based on colony morphology, haemolytic properties, CAMP reaction and fermentation of aesculine. They were grouped according to Lancefield's grouping system.

Data analysis

Herd level. From the annual samples, the herd prevalence of *Staph. aureus* or *Str. dysgalactiae* was calculated as the number of cows with these isolates in one or more quarters divided by the total number of cows sampled in the herd (Dohoo et al. 2003). Data were compiled using SAS software (version 8e). Multivariable models were constructed using PROC MIXED procedures with herd as the repeated observation. In herd model 1, the herd prevalence of *Staph. aureus* or *Str. dysgalactiae* each year was applied as dependent variable. A compound symmetry matrix was chosen according to the −2 log likelihood assessment of model fit after having explored the results of the autoregressive matrix, the compound unstructured matrix and the Toeplitz matrix. The independent fixed effects in the model were dry cow therapy (A, B or C), teat dipping regime (A, B or C), the annual sampling in the trial (0, 1, 2), interaction between dry cow therapy regime and annual sampling and the interaction between teat dipping and annual sampling. The interaction variables were always included in the model as long as dry cow therapy or teat dipping was included. These variables made sure that we analysed for the associations during the trial compared with the starting point. In herd model 2, separate models were made where the prevalence of *Staph. aureus* or *Str. dysgalactiae* after 1 year and 2 years were used as dependent variable. In these separate models, the prevalence of *Staph. aureus* or *Str. dysgalactiae* at the beginning of the study were used as independent fixed effects together with the interactions between this prevalence and both the dry cow therapy and the teat dipping regime. For herd models 1 and 2, the independent variables in the full models were excluded one by one with backward elimination procedure until all remaining variables were assessed as being significant or $P \leqslant 0\cdot10$.

In herd model 3, we checked for the differences in the random HERDID effect within each teat dipping group.

We used separate PROC MIXED models for each teat dipping group where annual sampling 1 and 2 were used as independent variables and HERDID as random effect.

Cow level. Dry cows and cows with missing samples were not included in the analysis. A positive *Staph. aureus* or *Str. dysgalactiae* cow was assessed as having an isolate of *Staph. aureus* or *Str. dysgalactiae* (1) if the bacteria were isolated from one or more quarters. Cows were classified as negative (0) if none of the quarters had any such isolate. The same procedure was used for *Str. dysgalactiae*. Multivariable models were fitted separately with *Staph. aureus* or *Str. dysgalactiae* positive cows or not as dependent variables. PROC GENMOD with binomial distribution and logit link function with Walds' statistics for Type 3 contrasts was used to assess significant contribution for each independent fixed effect.

The full model consisted of these independent fixed variables: parity (class 1, 2, 3 and >3), sine and cosine of calving day to evaluate any seasonal effect as descried by Østerås et al. (2006), the natural log of days in milk (lnDIM), dry cow therapy group (A, B or C), teat dipping group (A, B or C), yearly sampling number (0, 1 or 2), days from sampling to receipt at the mastitis laboratory (0, 1, 2, 3 or >3), geographical area (A, B, C, D, E or F) and finally the interaction between the teat dipping groups and the annual sampling. DIM was also tested in the model, instead of lnDIM, but lnDIM gave a better fit of the model. Cow identity (COWID) was included as a random effect nested within the herd identity (HERDID). The random effect was expressed as the log (OR) by using the LOGOR statement in the model as alternative logistic regression (Carey et al. 1993). Goodness of fit was assessed with deviance or Δ deviance in the different models. Variables were assessed as significant when $P \leqslant 0\cdot05$. The independent fixed effects in the full models were excluded one by one from the model with backward elimination procedure until all remaining variables were assessed as being significant or $P \leqslant 0\cdot10$.

Results

Of the 215 herds initially enrolled, 178 herds remained in the study after 2 years. Twelve farmers withdrew from the study because they did not manage the protocol or they suffered some accidents or *force majeure* outside this project. Another 23 farmers withdrew because their local veterinarian suspected that the protocol was not being followed or 50% or more of the milk samples were forgotten. Two farmers withdrew because their co-ownerships were dissolved during the study period. The distribution of the dry cow therapies and teat dips, together with herd descriptions are given in Table 1.

Results of the bacteriological findings, at quarter level, are summarized in Table 2. At quarter level, the proportional ratio of the different pathogens changed, with

Table 2. Bacterial isolates at quarter level during the trial. Figures in the first column each year represent percentages of quarter milk samples. In the second column, proportional rate of different pathogens are presented. First sample, at the beginning of the trial, is defined as year 0, then annual sampling during the trial after 1 and 2 years
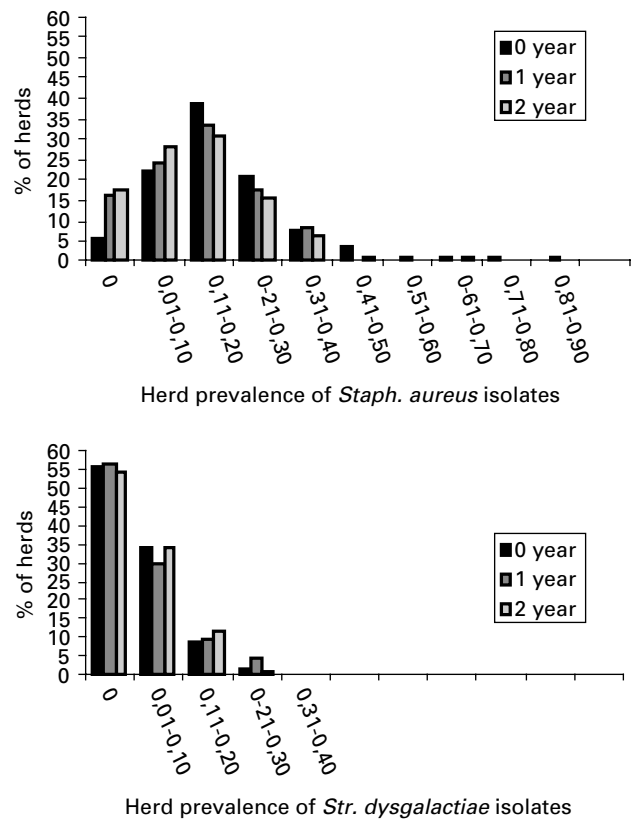
	0 year		1 year		2 years	
	% of total quarters $n=13\,798$	Proportional rate of positive quarters $n=1278$	% of total quarters $n=13\,372$	Proportional rate of positive quarters $n=1018$	% of total quarters $n=14\,004$	Proportional rate of positive quarters $n=1197$
Staphylococcus aureus	6·10	65·88	4·55	59·72	4·69	54·89
Streptococcus dysgalactiae	1·32	14·24	1·42	18·66	1·30	15·20
Streptococcus uberis	0·25	2·74	0·52	6·88	0·47	5·51
Escherichia coli	0·09	1·02	0·11	1·47	0·12	1·42
Coagulase negative staphylococci	1·47	15·89	0·96	12·57	1·81	21·22
Arcanobacterium pyogenes	0·02	0·23	0·01	0·10	0·10	0·25
Other bacteria	0·11	1·17	0·14	1·87	0·34	3·93
Dry quarters	1·76		1·87		1·75	
Contaminated samples	0·30		0·49		0·62	
No bacteria	88·58		89·93		88·88	
Total	100·00	100·00	100·00	100·00	100·00	100·00

less *Staph. aureus*, the same level of *Str. dysgalactiae* and an increased level of *Str. uberis* and coagulase negative staphylococci. There were no differences between the different dry cow therapy and teat dipping groups.

Herd level

The distribution of herd prevalence of *Staph. aureus* infected cows changed during the study period (Fig. 1). The prevalence of *Staph. aureus* at herd level, adjusted for the herd effect was 0·18 (±0·009) at the beginning, 0·14 (±0·009) after 1 year and 0·13 (±0·009) after 2 years. Altogether 19% of the variation in the herd level prevalence of *Staph. aureus* could be attributed to the herd. In the 178 herds, 15% of the herds showed no *Staph. aureus* positive cows after two years compared with 5% at the beginning. The distribution of herd prevalence of *Str. dysgalactiae* infected cows remained the same after 2 years (Fig. 1). The PROC MIXED herd model 1, with repeated measurements at herd level, revealed that there were no significant effects of either the different dry cow therapies, teat dipping regimes or any of the interactions between these two independent fixed effects and the annual sampling in the study. There were no significant associations between any of the independent fixed effects and the *Str. dysgalactiae* herd prevalence. In this model, 20% of the variation in the *Str. dysgalactiae* herd prevalence could be attributed to the herd.

In herd model 2, where *Staph. aureus* prevalence after 1 year and 2 years were used as the dependent variable and the *Staph. aureus* prevalence at the beginning of the study was used as independent variable, the only significant effect was the *Staph. aureus* prevalence at the beginning of the trial ($b=0·14±0·05$). There was no significant effect of the different dry cow therapies or teat

Fig. 1. The herd prevalence distribution of *Staphylococcus aureus* and *Streptococcus dysgalactiae* isolates at the beginning (year 0), after 1 year and after 2 years in the trial.

dipping regimes. However, in this model, 24% of the variation in the *Staph. aureus* herd prevalence could be attributed to the herd. Where the prevalence of *Str. dysgalactiae* after 1 and 2 years was used as a dependent

Table 3. Estimates for the final logistic regression model (PROC GENMOD), at cow level, where cows were assessed as having an isolate of *Staphylococcus aureus* (yes=1 or no=0) and *Streptococcus dysgalactiae* (yes=1 or no=0) were used as dependent variable. Only annual samplings 1 and 2 are included

Independent fixed and random effects	Parameter	n	*Staphylococcus aureus* n(0)=5834, n(1)=942 Estimate	SE	OR† (95 % CI)	P-value	*Streptococcus dysgalactiae* n(0)=6480, n(1)=296 Estimate	SE	OR (95 %CI)	P-value
	Intercept	6776	−1·07	0·15		<0·001	−4·24	0·37		<0·001
Parity	1	2861					−1·16	0·19	0·31 (0·21–0·45)	<0·001
	2	1846					−0·34	0·15	0·71 (0·53–0·96)	<0·05
	3	1123					−0·09	0·20	0·91 (0·62–1·35)	NS
	>3	946					0	0	—	—
lnDIM‡		6776	−0·17	0·03	0·84 (0·80–0·89)	<0·001	−0·15	0·06	0·86 (0·77–0·97)	<0·05
Fixed										
Teat dipping	A (Negative)	2913					0·43	0·18	1·54 (1·08–2·19)	<0·05
	B (iodine)	1842					0	0	—	—
	C (DryFlex™)	2021					0·59	0·20	1·80 (1·22–2·67)	<0·01
Geographical areas	A	1367					0·90	0·29	2·46 (1·39–4·34)	<0·01
	B	1445					1·20	0·27	3·32 (1·96–5·64)	<0·001
	C	1343					1·13	0·28	3·10 (1·79–5·36)	<0·001
	D	739					0·44	0·34	1·55 (0·80–3·02)	NS
	E	656					−0·87	0·47	0·42 (0·17–1·05)	NS
	F	1226					0	0	—	—
Random§										
Within cow	Alpha 1		0·92	0·18	2·51 (1·76–3·57)	<0·001	1·09	0·37	2·97 (1·44–6·14)	<0·01
Within herd	Alpha 2		0·21	0·04	1·23 (1·14–1·33)	<0·001	0·21	0·07	1·23 (1·08–1·42)	<0·01

† Odds ratio with 95 % confidence intervals
‡ natural log of days in milk, mean days in milk 130 d ±92
§ Number of clusters=178, minimum cluster size 9; maximum cluster size 94
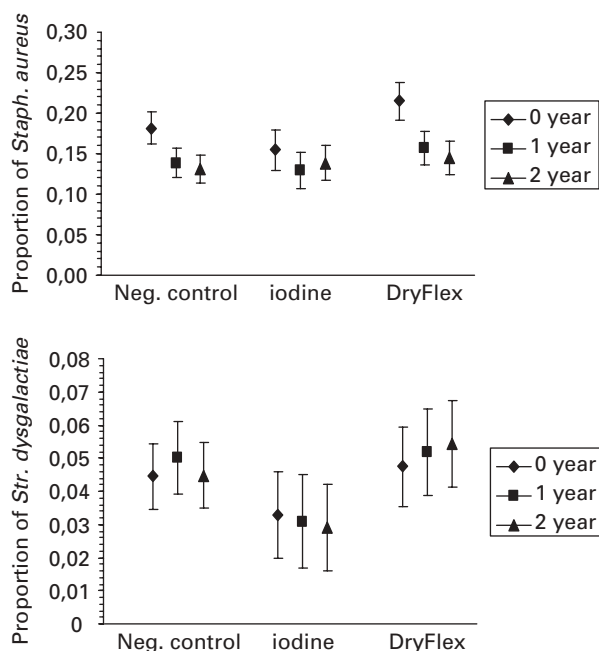
Fig. 2. Crude cow level proportion with confidence interval, *Staphylococcus aureus* or *Streptococcus dysgalactiae* positive cows divided by total number of sampled cows within each teat dipping regimes at the beginning (year 0), after 1 year and after 2 years in the trial.

variable, the only significant independent fixed effect identified was the prevalence of *Str. dysgalactiae* at the beginning of the study (b=0·27±0·07).

Herd model 3, the separate PROC MIXED models for each of the teat dipping group, revealed a herd effect of 38% for group A (control), 28% for group B (iodine) and 11% for group C (DryFlex™) for *Staph. aureus* and 12% for group A, 0% in group B and 22% in group C for *Str. dysgalactiae*.

Cow level

At cow level, there were no significant differences in the reduction of *Staph. aureus* between the different teat dipping regimes (Fig. 2). In the PROC GENMOD models, the classification 'Staph. aureus cow' or not, from the annual sampling 1 and 2, was used as dependent variable. The only significant independent fixed effect was lnDIM (Table 3). In the same model, but with 'Str. dysgalactiae cow' or not as dependent variable, there was a significant effect of parity, lnDIM, teat dipping (B *v.* A or C) and some geographical areas. Results are presented in Table 3.

Discussion

We observed a reduction of *Staph. aureus* after 2 years in the trial. This is due to the implementation of the selective dry

cow therapy protocol and the selective culling strategy of cows with a long history of high CMSCC. This is in accordance with the results of Østerås et al. (1991) who conducted a similar study where a negative control group was included. They found a significant decrease in subclinical *Staph. aureus* mastitis in cows using dry cow therapy compared with a negative control group. They used the same dry cow therapy formula as Juvanesta (300 000 i.u. benzylpenicillin and 300 mg dihydrostreptomycin) which served as a positive control in our trial, and justifies the lack of a negative control (Dohoo et al. 2003).

The reason why we did not observe a reduction in *Str. dysgalactiae* after 2 years is unknown and was unexpected. We should expect a therapeutic effect of the three different selective dry cow therapies close to 100%, which would be in accordance with Østerås et al. (1991). *Str. dysgalactiae* mastitis is an emerging problem in Norway, causing more clinical and subclinical mastitis than in previous years (Norwegian Cattle Health Service, Annual Report 2004). Little research has been conducted on *Str. dysgalactiae*, a microbe which may be contagious in some dairy herds and environmental in other herds (International Dairy Federation, 1999).

Our present results demonstrate that neither iodine dip nor DryFlex™ had any effect on reducing the prevalence of *Staph. aureus* or *Str. dysgalactiae* at herd level. At cow level, we found a significantly higher odds ratio (OR) of *Str. dysgalactiae* positive cows when comparing the DryFlex™ group with the iodine group (OR=1·5, CI 95% 1·1 to 2·2) and the negative control with the iodine group (OR=1·8, CI 95% 1·2 to 2·7). The lack of improvement in prevalence and in occurrence of *Staph. aureus* at cow level are contradictory to other iodine teat dipping trials, at cow or quarter level, where udders have been either experimentally or naturally exposed to *Staph. aureus*. One explanation for these results could be that the present trial was been conducted at herd level in 178 commercial dairy farms where other herd effects may play an important role in reducing the prevalence of *Staph. aureus*. A DryFlex™ trial is not approved by the NMC protocol, and as far as we are concerned, DryFlex™ has never been tested in large field trials where the included herds have a high proportion of *Staph. aureus* and *Str. dysgalactiae* isolates and the reason for including DryFlex™ was to see whether it had an impact on *Staph. aureus* and *Str. dysgalactiae* infected herds. There have been many efficacy studies conducted on DryFlex™ in research herds, but very few with published data and only one trial mentions *Staph. aureus*, which was a natural exposure trial conducted in Argentina. The results showed that DryFlex™ was especially effective against environmental streptococci and coliforms, but no differences were observed between the two groups in reducing *Staphylococcus aureus* infections (Corbellini et al. 2002).

The significant variables in the PROC GENMOD models (at cow level) were lnDIM (*Staph. aureus* model) and lnDIM and some geographical areas (*Str. dysgalactiae*).

The choice of lnDIM, instead of DIM, was based on better fit of the model using lnDIM, which tells us that both *Staph. aureus* and *Str. dysgalactiae* positive isolates are associated with samples taken close to calving compared with later in the lactation. These findings are partly in accord with Østerås et al. (2006) who found more *Staph. aureus* positive samples after calving compared with late lactation, but *Str. dysgalactiae* positive isolates increased in late lactation. Our results also tell us that there are differences between the different geographical areas with regard to the prevalence. The farmers and the veterinarians' effort and expertise in taking samples, treating and culling correct cows are one factor influencing these differences.

The effect of teat dipping over time was carefully looked into by including the interaction between teat dipping and the two annual samplings in herd model 1. It is important to look at the interaction between the prevalence and the teat dipping over time since this interaction will illustrate any increase or decrease related to teat dipping as the trial progresses. The effect of dry cow therapy over time was not significant and was excluded from the model after dry cow therapy was excluded. Since the prevalence of *Staph. aureus* and *Str. dysgalactiae* differed between the three teat dipping groups at the beginning of the trial (Fig. 2), the first annual sampling (year 0) was included as an independent variable in separate models to adjust for different starting positions.

In herd model 3, the random herd effect of *Staph. aureus* seems to be higher for the control group and lower for the DryFlex™ group. Higher random herd effects demonstrate a higher correlation between the herd prevalence in year 1 and year 2 in the trial. For *Str. dysgalactiae*, this clustering turned out to be zero in the iodine group. This means there is no correlation between the prevalence in the herds in year 1 and in year 2, which indicates that it would be difficult to control *Str. dysgalactiae* in these herds with current knowledge as the mean prevalence stays constant.

In the present study, the herd effect was taken care of by randomly allocating the herds independently into the different dry cow therapies or teat dipping regimes. However, herd level studies could be biased by any herd effect. In statistical analysis at cow level, the herd effect may overestimate the significance of the responsible variable as the variation usually will be underestimated (McDermott et al. 1994). It is therefore important to include this effect as a random term.

The present results give no indication that the prevalence of *Staph. aureus* in Norwegian herds could be significantly decreased by a general recommendation for teat dipping with iodine or application of DryFlex™ as done in this study. However, they do indicate that iodine teat dipping could be beneficial in herds with a high prevalence of *Str. dysgalactiae* positive cows as demonstrated by a significant effect of iodine teat dip at cow level. The zero cluster effect at herd level for iodine teat dip also indicates

that there are some additional new infection mechanisms that have to be controlled, despite using iodine teat dip.

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