



An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom

A. J. Bradley,*†¹ J. E. Breen,*† B. Payne,* V. White,* and M. J. Green†

*Quality Milk Management Services Ltd., Cedar Barn, Easton Hill, Easton, Nr Wells, Somerset, BA5 1DU, United Kingdom

†University of Nottingham, School of Veterinary Medicine and Science, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, United Kingdom

ABSTRACT

Vaccination can play a useful role in mastitis control programs, although there is a relative dearth of large, well-controlled field efficacy studies. This paper presents the findings on the use of a commercially available vaccine (Startvac, Hipra UK Ltd., Nottingham, UK) on commercial units under UK field conditions. In total, 3,130 cows were recruited from 7 farms and were randomly allocated, within farm, to 1 of 3 groups. The first group received the vaccine following the label regimen, the second group was vaccinated every 90 d following an initial vaccination course, and the third group was left unvaccinated to act as controls. Vaccine efficacy was assessed in the first 120 d of lactation. Data were available for analysis from 1,696 lactations in 1,549 cows. In total, 779 cases of clinical mastitis occurred in the 3 study groups, and we detected no significant difference in the incidence or prevalence of clinical or subclinical mastitis between any of the 3 groups. Mastitis vaccination following the label regimen was associated with a significant reduction in the severity of clinical cases. Cows in this group were at significantly decreased odds of developing clinical mastitis presenting with more than just milk changes [odds ratio: 0.58; 95% confidence interval (CI): 0.35–0.98]. Similarly, each additional vaccination resulted in a cow being at decreased odds of developing clinical mastitis presenting with more than just milk changes (odds ratio: 0.87; 95% CI: 0.77–0.98). Although no cows were culled because of severe mastitis in either of the vaccinated groups, we detected no significant difference in the mastitis-related culling rate between groups. Analysis of milk production data demonstrated that, on average, cows on the label regimen produced a higher volume of milk (231 L; 95% CI: 104.1–357.4) and more milk solids (12.36 kg; 95% CI: 3.12–21.60) than unvaccinated cows in the first 120 d of lactation. Conservative analysis suggested that a return

on investment of 2.57:1 could be expected under UK conditions based on increased milk yield alone.

Key words: vaccination, mastitis, milk production, coliform, *Escherichia coli*

INTRODUCTION

Clinical and subclinical mastitis remain a major cause of financial loss to the dairy industry and a significant challenge to the dairy producer, with a large number of herds still experiencing unacceptable levels of disease (Bradley et al., 2007b). Several treatments and control measures are available to the practitioner but these are often apparently insufficient to control the disease on farm (Green et al., 2007).

Effective mastitis vaccination has long been the “holy grail” of mastitis control. However, despite development of several vaccines in the 1980s, based on the J5 *Escherichia coli* mutant, such vaccines to date, although demonstrating an ability to reduce the severity of clinical signs and duration of infection, have failed to demonstrate a reduction in the rate of IMI (Hogan et al., 1992; Wilson et al., 2007a). Investigation of the use of J5 coliform vaccines has also demonstrated a positive effect on production in that vaccinated cows have been shown to recover milk yield after a clinical case more quickly than unvaccinated cows (Wilson et al., 2007b, 2008, 2009).

Although mastitis vaccines have been available in many jurisdictions, this has not been the case in the European Union until relatively recently. However, a polyvalent mastitis vaccine directed against both enterobacterial and staphylococcal species has been approved for use in the European Union (Startvac; Hipra UK Ltd., Nottingham, UK). Registration studies demonstrated a reduction in IMI with coliform and *Staphylococcus* spp. and a decrease in severity of clinical signs of disease when using the product (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/veterinary/000130/WC500068576.pdf). However, these registration studies were based primarily in southern Europe and were conducted under very different climatic and management

Received May 6, 2014.

Accepted October 9, 2014.

¹Corresponding author: Andrew.Bradley@qmms.co.uk

conditions to those seen in northern Europe and the United Kingdom.

A significant constraint to the use of mastitis vaccines has been the relatively onerous vaccination regimens (Wilson and González, 2003) that are necessary to achieve the desired level of efficacy. These often necessitate vaccination both before and after calving (González et al., 1989). This has led to the development of more practical, farmer-friendly approaches to vaccination when J5 core antigen vaccines have been deployed in the field, such as a rolling schedule of vaccination of all cows in the herd on a quarterly basis. Other attempts at improving efficacy have also been made by increasing the number of vaccinations (Erskine et al., 2007) and by vaccinating earlier in the lactation cycle (Gurjar et al., 2013), in part to reduce the effect of IMI acquired during the dry period (Bradley and Green, 2000).

The aim of the study outlined here was to investigate the efficacy of a multivalent mastitis vaccine (Startvac; Hipra UK Ltd.) in the control of bovine mastitis under UK field conditions using both the label regimen and a schedule of quarterly vaccination.

MATERIALS AND METHODS

Herd Selection

Seven commercial dairy herds, in the southwestern United Kingdom, were selected to participate in the study based on location, likelihood of compliance with the study protocol, suitable herd records, a suitably maintained milking machine, and enrollment in regular DHI testing. No strict criteria were applied pertaining to bulk milk SCC or clinical mastitis incidence. Each herd was visited by a veterinarian to provide suitable training to ensure study compliance.

Cow Selection

All cows and heifers approaching their first calving were eligible for recruitment to the study, contingent on being in good health, having 4 functional quarters, teats free of significant teat lesions, and an estimated calving date to allow vaccination at predicted times before calving.

Vaccine Selection

The vaccine selected for use in this study (Startvac; Hipra UK Ltd.) was a polyvalent product containing inactivated *Escherichia coli* J5 and inactivated *Staphylococcus aureus* (CP8) strain SP 140, expressing slime-associated antigenic complex (SAAC), utilizing a liquid

paraffin adjuvant and containing benzyl alcohol as an excipient.

Study Protocol

Enrollment. Farms were initially visited and all lactating and nonlactating adult cows present on the farm were recruited to the study. Herd personnel were trained to maximize compliance with the study protocol. Training encompassed the identification, scoring, and aseptic sampling of clinical mastitis and the use of the California Mastitis Test. Thereafter, each site was visited weekly to allow recruitment of heifers and purchased cows joining the herd as well as the collation of farm records, sample collection, reinforcement of training, and the administration of vaccinations as outlined below.

Treatment Allocation and Administration. At the initial visit to each site, all eligible cows were randomly allocated to 1 of the 3 study groups; namely, unvaccinated, label, and rolling regimen groups. Thereafter, cows joining the herd were also randomly allocated to 1 of the 3 study groups. Heifers were recruited if service dates had been accurately recorded. Once enrolled, cows remained in the same treatment group for subsequent lactations. All vaccinations were administered by study personnel.

Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label and rolling groups were vaccinated (by deep intramuscular injection) according to the schedule outlined below. The study was conducted under field conditions and, therefore, vaccinations were carried out weekly, with all vaccinations due in the next 7 d being undertaken at each visit (i.e., cows due to be vaccinated 45 d before calving may have been vaccinated between 52 and 45 d before calving).

Rolling Vaccination Regimen. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study. New cows entering the herd were vaccinated at the earliest opportunity and followed the same regimen, although they received their vaccinations at a different time from the majority of cows that entered this regimen.

Label Vaccination Regimen. Cows recruited to the label group were not vaccinated on enrollment but were monitored and subsequently vaccinated according to the licensed regimen at 45 d before the estimated date of calving (based on herd records), 35 d later (10 d before the estimated date of calving, although this could actually be postcalving if cows calved early), and 52 d postcalving. Label regimen cows were not vaccinated again until 45 d before their next estimated calving date.

Masking and Bias-Reducing Methods. It was not possible to completely blind study or farm personnel to product administration because no placebo was administered. However, the potential for bias was reduced by the use of randomization tables in the allocation of cows to treatment group and because no form of identification was used that allowed visual identification of cows based on their treatment group. Moreover, although it was possible for bias to be introduced in the subjective identification of clinical mastitis, other outcomes used in the study such as yield and SCC could not be influenced by study personnel. Laboratory personnel undertaking somatic cell counting and clinical mastitis bacteriology were completely blind to product administration.

Clinical Mastitis and Production Monitoring. Farm personnel monitored cows for the presence of clinical mastitis throughout the study period and collected a pretreatment aseptic quarter milk sample when cases occurred. These samples were frozen on farm and stored until the next routine visit. All personnel were trained in detection, grading, and aseptic sampling of clinical mastitis following standard operating procedures. Clinical mastitis cases were scored for clinical severity (grade 1 = milk changes only; grade 2 = milk or udder changes or both; grade 3 = signs of systemic disease (e.g., loss of appetite, change in demeanor, elevated rectal temperature, $>39.2^{\circ}\text{C}$) with or without milk or udder changes; grade 4 = signs of severe depression and toxemia or toxic shock).

Individual cow production was monitored through an International Committee for Animal Recording (ICAR)-accredited milk recording scheme run by QMMS Ltd. (Somerset, UK).

Laboratory Procedures

All milk samples collected were maintained at or below 8°C during transport to the laboratory for analysis. Microbiological investigation and SCC were carried out using the standard milk sample examination techniques, which exceeded the standard recommended by the International Dairy Federation (Bulletin No 132), International Standard 13366-1:1997 (E) and 13366-2:1997 (G; International Dairy Federation, 1981). A more complete description of these techniques is outlined below.

Bacteriology. Ten microliters of secretion was inoculated onto sheep blood agar and Edward's agar, and 100 μL of secretion was inoculated onto MacConkey agar to enhance the detection of *Enterobacteriaceae*. Plates were incubated at 37°C and read at 24, 48, and 72 h. Organisms were identified and quantified using standard laboratory techniques (Quinn et al., 1994; Na-

tional Mastitis Council, 1999) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; MALDI Biotyper, Bruker Daltonics, Billerica, MA).

The primary method used to identify organisms was MALDI-TOF MS. Individual colonies selected for the purposes of identification were applied to a steel plate (Bruker Daltonics) and allowed to dry at room temperature before overlay with 1 μL of MALDI Matrix [a saturated solution of α -cyano-4-hydroxycinnamic acid (Bruker Daltonics) in 50% acetonitrile and 2.5% trifluoroacetic acid]. Spectra were generated using the manufacturer's suggested settings and captured and analyzed using the flexControl and MALDI Biotyper 3.0 software (Bruker Daltonics). Ions were generated with a 337-nm nitrogen laser and captured in the positive linear mode in a mass range of 2 to 20 kDa. Spectra were compared with a database containing in excess of 3,500 spectra from over 2,000 bacterial species. Each plate also carried a standard (Bacterial Test Standard, Bruker Daltonics) to calibrate the instrument and validate each run. Organisms were identified using criteria outlined by the manufacturer; this method uses an integrated pattern-matching algorithm to match spectral peaks against known bacterial species before assigning a log score against a maximum value of 3.0. For the purposes of this study, identifications were only accepted where a score >2.0 was achieved and a "probable" species-level identification applied that was also consistent with colony and organism morphological characteristics. In the very small number of cases in which a species-level identification could not be achieved, conventional biochemical techniques were used such as API (bioMérieux, Basingstoke, UK).

Somatic Cell Counting. Samples were collected into vials containing an 18-mg tablet containing 8 mg of bronopol and 0.30 mg of natamycin (Broad Spectrum Microtabs II, Advanced Instruments Inc., Norwood, MA). The SCC was determined using the Fosomatic method (Delta CombiScope, model FTIR 400, Drachten, the Netherlands), according to the FIL-IDF 148 A: 95 norm (International Dairy Federation, 1995).

Assessment of Efficacy

For the purposes of the analysis outlined in this paper, efficacy of the different vaccination regimens was compared with no vaccination in the first 120 d of lactation. This period was selected because it corresponded to the period of expected efficacy when following the label vaccination regimen. To conduct the analysis, a suitable population of cows had to be selected from among those recruited to the study to ensure sufficient time had elapsed following vaccination for a "protective" im-

immune response to have been raised and to ensure groups could be compared in a contemporaneous manner. This was achieved using the following process. Label cows were included if they had received at least 2 vaccinations before calving. Rolling cows were included in the analysis if they had received at least 2 vaccinations before calving. In both vaccinated groups, the time before calving of the second vaccination was controlled for as a potentially confounding factor. Unvaccinated cows were only recruited if sufficient time had elapsed since enrollment for them to have received 2 vaccinations had they been in either of the other treatment groups, thereby ensuring all 3 groups were temporally matched.

Clinical Mastitis. Both overall and pathogen-specific clinical mastitis rates were investigated in the first 120 d of lactation. The effect of vaccination on clinical mastitis severity was assessed using all cases of clinical mastitis occurring in the first 120 d lactation.

SCC. The effect of vaccination on SCC was assessed in several ways, primarily by investigating the effect on SCC at each of the first 4 individual recordings in lactation (as long as they occurred with 120 d of calving). First, second, third, and fourth test-days were defined by their timing during lactation, occurring at 5 to 30, 31 to 60, 61 to 90, and 91 to 120 d in milk, respectively. In addition, using SCC as a proxy for infection status and a threshold of 200,000 cells/mL, the apparent prevalence of infection at the first recording (within 30 d of calving) was assessed. In addition, the apparent dry period cure and dry period new infection rates were calculated, based on movements around the 200,000 cells/mL threshold, using the last SCC before dry off and the first SCC postcalving (Green et al., 2008). Finally, the apparent rate of new infection during lactation in the first 120 d of lactation was assessed using movements around the same SCC threshold between successive dairy herd improvement tests (Bradley et al., 2007a).

Milk Yields. The effect of vaccination on milk yield and milk solids production was assessed using data available from DHI tests. Milk production in the first 120 d of lactation was calculated using the test interval method (http://www.icar.org/documents/Rules%20and%20regulations/Guidelines/Guidelines_2012.pdf).

Culling. The overall culling rates as well as the mastitis-specific culling rates were calculated in the first 120 and 305 d of lactation.

Data Collation and Statistical Analyses

Data were collated and initially analyzed using Excel and Access 2003 (Microsoft Corp., Redmond, WA) and Minitab 15.1 (Minitab Inc., State College, PA). Descriptive and graphical analyses were carried out to explore the data. When appropriate, groups were

compared using ANOVA or, if data were not normally distributed, the Kruskal-Wallis test. Univariable analysis of treatment efficacy was performed using the chi-square test to investigate differences in proportions between groups; a layered Bonferroni correction was used to allow for multiple comparisons where appropriate (Darlington, 1990).

When assessing the efficacy of the different regimens in the control of clinical mastitis and SCC and the effects on milk yield and culling, conventional multilevel (random effects) models (Goldstein, 1995) were specified so that correlations within the data were accounted for appropriately. General model specifications (for the binary outcomes) were as follows:

$$Y_{ij} \sim \text{Bernoulli}(\text{probability} = \pi_{ij}),$$

$$\text{Logit}(\pi_{ij}) = \alpha + \beta_1 \mathbf{X}_{ij} + \beta_2 \mathbf{X}_j + u_{jk},$$

$$u_{jk} \sim N(0, \sigma_u^2),$$

where Y is the outcome under consideration; the subscripts i and j denote the i th quarter and the j th cow, respectively; α is the regression intercept; \mathbf{X}_{ij} is the vector of covariates at quarter level and β_1 the coefficients for covariates \mathbf{X}_{ij} ; \mathbf{X}_j is the vector of farm-year level covariates and β_2 the coefficients for covariates \mathbf{X}_j ; u_{jk} is the random effect to reflect residual variation between cows; and σ_u^2 = between-cow variance. Farm was included as a fixed effect in all models and a term for \log_e week of study was included to account for the underlying risk of mastitis over time. Equivalent models with normally distributed outcome variables were specified to separately investigate the effects of vaccination routine on milk yield and milk solids.

Covariate assessment and selection was carried out using MLwiN with MQL (marginal quasi likelihood) or PQL (penalized quasi likelihood) for parameter estimation (Rasbash et al., 2005). A significance probability was set at $P < 0.05$. Investigation of model fit was using standard methods as previously described (Goldstein, 1995; Green et al., 2004).

Survival analysis was used to examine factors that influenced the risk of clinical mastitis or culling in the first 120 d of lactation. This was conducted using MLwiN following conventional methods, with the introduction of a lowest level of time (Collett, 1995).

RESULTS

In total, 3,130 cows from 7 farms were recruited to the study between September 2010 and January 2012; key characteristics of each of each of the farms and

Table 1. Key characteristics of the 7 study farms

Characteristic	Study site						
	B	C	F	H	P	R	T
Herd size	190	568	218	231	286	205	581
Number of animals recruited	240	780	274	307	421	284	824
Bulk milk SCC ¹ ($\times 10^3$ cells/mL)							
1 mo before study	254	238	288	193	356	349	260
2 mo before study	235	229	285	223	377	286	269
3 mo before study	404	298	295	260	211	306	254
At the end of study	217	468	283	201	288	526	310
Clinical mastitis incidence rate (cases/100 cows per year)							
In 12 mo before start of the study	36	111	67	114	40	149	41
In 12 mo before the end of the study	82	38	55	101	86	147	48
305-d milk yield (L)							
At the start of the study (cows and heifers)	8,843	9,280	9,918	9,012	8,917	8,758	10,654
At the end of the study (cows and heifers)	8,454	10,352	9,401	9,547	8,753	8,725	10,542
Predominant housing ²							
Dry cow–winter	Y	C, Y	C, Y	Y	C, Y	C, Y	C, Y
Lactating cow–winter	C	C	C	C	C	C	C
Dry cow–summer	P	P	C, Y	P	P	P	Y
Lactating cow–summer	P	C	C	P	P	P	C
Predominant bedding							
Dry cows	Straw	Sand	Sand	Straw	Straw	Straw	Straw
Lactating cows	Straw	Straw	Sand	Sand	Straw	Straw	Straw
Milking frequency (/d)	2 \times	3 \times	2 \times	2 \times	2 \times	2 \times	3 \times

¹Calculated bulk milk SCC based on individual cow recordings.

²C = cubicles; Y = yards; P = pasture.

the number of cows recruited from each are shown in Table 1. In total, 1,044, 1,046, and 1,040 cows were allocated to the unvaccinated, label, and rolling groups, respectively. Data from a total of 1,549 cows that calved (or would have calved in the case of the unvaccinated group) after 2 or more vaccinations were incorporated into the analyses; 576, 415, and 558 cows in the unvaccinated, label, and rolling groups, encompassing 642, 416, and 638 lactations, respectively. As stipulated by the selection criteria above, cows in the label group had received 2 vaccinations by calving, whereas cows in the rolling group had received, on average, 4.26 (minimum = 2, maximum = 7) vaccinations by calving. Key characteristics of cows used in the analysis of efficacy

are outlined in Table 2 and did not vary significantly between the study groups.

Clinical and Subclinical Mastitis

In total, 4,237 cases of clinical mastitis occurred in all cows on the study farms during the study period, of which 779 occurred in the first 120 d of lactation in cows eligible for analysis of vaccine efficacy in early lactation. The etiology of clinical mastitis in cows on the study was complex (summarized in Table 3). *Escherichia coli* was the most common cause of clinical mastitis, accounting for approximately one-fifth of cases, whereas all *Enterobacteriaceae* accounted for approxi-

Table 2. Summary of the key data from cows included in the analysis of efficacy in early lactation¹

Item	Unvaccinated group			Label group			Rolling group		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
Parity	3.72	3	1–14	3.57	3	1–12	3.62	3	1–13
Last recorded yield before dry off (L)	18.3	17.7	1.5–51.6	18.0	17.6	2.8–43.9	18.2	17.4	1.2–50.6
ICSCC ² ($\times 10^3$ cells/mL)									
1 mo before dry off	371	174	12–8,062	301	121	6–4,440	384	181	10–8,486
2 mo before dry off	269	121	11–6,771	244	122	4–5,333	277	143	8–5,267
3 mo before dry off	255	108	9–6,602	227	107	8–6,883	259	115	6–9,203

¹Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were vaccinated at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study.

²ICSCC = individual cow SCC.

mately one-quarter of cases, and gram-negative organisms were implicated in approximately one-third of all clinical cases. Clinical cases caused by *Staphylococcus aureus* and the CNS were relatively rare, accounting for only 2.5 and 5.6% of cases, respectively.

The proportion of cows affected and rates of clinical mastitis in the first 120 d of lactation are summarized in Table 4. Survival curves for the overall occurrence of clinical mastitis in each group are illustrated in Figure 1. Univariable and multivariable analysis failed to identify any significant differences in pathogen-specific or overall rates of clinical mastitis between the treatment groups. More specifically, when compared with unvaccinated cows, cows on the label and rolling regimens were no less likely to develop clinical mastitis [label regi-

men: odds ratio (OR) 0.91, 95% CI: 0.72–1.15; rolling regimen: OR 0.93, 95% CI: 0.76–1.14], coliform mastitis (label regimen: OR 1.22, 95% CI: 0.84–1.78; rolling regimen: OR 1.09, 95% CI: 0.77–1.53), staphylococcal mastitis (label regimen: OR 0.79, 95% CI: 0.54–1.16; rolling regimen: OR 0.88, 95% CI: 0.63–1.22), or clinical mastitis caused by a coliform or staphylococcal organism (label regimen: OR 1.04, 95% CI: 0.76–1.43; rolling regimen: OR 0.95, 95% CI: 0.71–1.26).

Somatic cell scores did not vary significantly ($P > 0.05$) between the treatment groups at any of the test-days during the first 120 d of lactation (see Table 5). The proportion of cows affected by subclinical mastitis and the rate of apparent new subclinical infection, as defined by an individual cow SCC >200,000 cells/mL

Table 3. Summary of the etiology of clinical cases included in the analysis of efficacy in early lactation¹

Item	Unvaccinated		Label group		Rolling group		Overall	
	n	%	n	%	n	%	n	%
Number of cows	576		415		558		1,549	
Number of lactations	642		416		638		1,696	
<i>Escherichia coli</i>	58	19.86	42	21.54	60	20.55	160	20.54
<i>Streptococcus uberis</i>	61	20.89	33	16.92	61	20.89	155	19.90
<i>Staphylococcus aureus</i>	7	2.40	5	2.56	7	2.40	19	2.44
<i>Enterococcus</i> spp.	4	1.37	6	3.08	8	2.74	18	2.31
<i>Streptococcus dysgalactiae</i>	9	3.08			7	2.40	16	2.05
Yeast spp.	3	1.03	5	2.56	6	2.05	14	1.80
<i>Bacillus</i> spp.	6	2.05	2	1.03	3	1.03	11	1.41
<i>Trueperella pyogenes</i>	5	1.71			6	2.05	11	1.41
<i>Enterobacter</i> spp.	3	1.03	1	0.51	2	0.68	6	0.77
<i>Klebsiella</i> spp.	2	0.68	1	0.51	2	0.68	5	0.64
<i>Serratia</i> spp.			3	1.54	2	0.68	5	0.64
<i>Lactococcus</i> spp.	1	0.34			3	1.03	4	0.51
<i>Pseudomonas</i> spp.	1	0.34	2	1.03	1	0.34	4	0.51
<i>Proteus</i> spp.			2	1.03	1	0.34	3	0.39
<i>Aerococcus</i> spp.	2	0.68					2	0.26
<i>Acinetobacter</i> spp.	1	0.34			1	0.34	2	0.26
<i>Aspergillus</i> spp.	1	0.34	1	0.51			2	0.26
<i>Micrococcus</i> spp.	1	0.34			1	0.34	2	0.26
<i>Streptococcus</i> spp.	1	0.34			2	0.68	2	0.26
<i>Prototheca</i> spp.			2	1.03			2	0.26
<i>Candida</i> spp.			1	0.51			1	0.13
<i>Citrobacter</i> spp.			1	0.51			1	0.13
<i>Gemella</i> spp.			1	0.51			1	0.13
<i>Pasteurella</i> spp.			1	0.51			1	0.13
<i>Staphylococcus</i> spp.			1	0.51			1	0.13
Unspecified gram-negative					1	0.34	1	0.13
All <i>Enterobacteriaceae</i>	63	21.58	50	25.64	67	22.95	180	23.11
Mixed etiology	35	11.99	33	16.92	31	10.62	99	12.71
Mixed with gram-negative involvement	29	9.93	20	10.26	23	7.88	72	9.24
Coagulase-negative <i>Staphylococcus</i> spp.	17	5.82	8	4.10	19	6.51	44	5.65
<i>Corynebacterium</i> spp.	6	2.05	6	3.08	13	4.45	25	3.21
Mixed minor pathogens	3	1.03	4	2.05	2	0.68	9	1.16
Contaminated	13	4.45	11	5.64	21	7.19	45	5.78
No growth	26	8.90	15	7.69	22	7.53	29	3.72
No sample	26	8.90	8	4.10	10	3.42	43	5.52
Total	292		195		292		779	
Cases/100 cow-lactations	45.5		46.9		45.8		45.9	

¹Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study.

Table 4. Summary of the proportion of cows affected and rates of clinical mastitis, caused by pathogens incorporated in the vaccine, in the first 120 d of lactation in the 3 treatment groups¹

Item	Unvaccinated group		Label group		Rolling group	
	Proportion of cows affected	Cases/100 cow-lactations	Proportion of cows affected	Cases/100 cow-lactations	Proportion of cows affected	Cases/100 cow-lactations
Number of cows		576		415		558
Number of lactations		642		416		638
All cases	0.334	45.5	0.321	46.9	0.332	45.8
<i>Escherichia coli</i>	0.098	9.5	0.116	7.9	0.113	9.6
Coliforms	0.112	9.8	0.128	12.0	0.124	10.5
<i>Staphylococcus aureus</i>	0.013	1.1	0.020	1.2	0.017	1.1
Coagulase-negative <i>Staphylococcus</i> spp.	0.069	2.7	0.064	1.9	0.062	3.0
All <i>Staphylococcus</i> spp.	0.078	3.7	0.081	3.4	0.071	4.1
All coliforms and <i>Staphylococcus</i> spp.	0.167	13.6	0.175	15.4	0.167	14.6

¹Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were vaccinated at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study.

at any of the DHI tests in the first 120 d of lactation, were not significantly different between the treatment groups.

Mastitis Severity and Culling

The effect of vaccination on clinical mastitis severity was assessed using all cases of clinical mastitis occurring in the first 120 d lactation; severity grades were available for 770 cases. The number and proportion of clinical mastitis cases falling into each of the severity classifications are outlined in Table 6. Univariable

analysis failed to demonstrate any significant difference in the proportion of cases falling into each severity classifications between treatment groups.

Multivariable analysis taking into account confounding factors such as mastitis etiology, parity, and farm effects demonstrated a significant effect of vaccination, with cows on the label regimen being at significantly decreased odds of developing clinical mastitis manifesting with more than just changes in the milk aspect (OR 0.58, 95% CI: 0.35–0.98). Details of the model are outlined in Table 7. Similarly, multivariable analysis demonstrated that an increasing number of vaccinations decreased the severity of clinical signs, as outlined in Table 8, with each additional vaccination resulting in a cow being at decreased odds of developing clinical mastitis manifesting with more than just changes in the milk aspect (OR 0.87, 95% CI: 0.77–0.98). These models also demonstrated that cases of clinical coliform mastitis were more likely to be severe than cases caused by other pathogens and that large variation in severity existed between study farms.

The overall culling rates and the mastitis-specific culling rates were calculated for the first 120 and 305 d of lactation, the findings of which are summarized in Table 9. Rates of culling were low, with only 14 (2.2%), 6 (1.4%), and 15 (2.4%) of cows being culled in the unvaccinated, label, and rolling groups, respectively. No significant effects of vaccination were detected. However, there were proportionally fewer mastitis culls in the label-vaccinated group, and the only toxic and fatal cases of mastitis occurred in the unvaccinated group. Because farmers tend to be reluctant to cull cows in early lactation, our analysis of the effect of vaccination on culling was extended to encompass the first 305 d of lactation. This revealed a significant difference in the total number of cows culled between the treatment

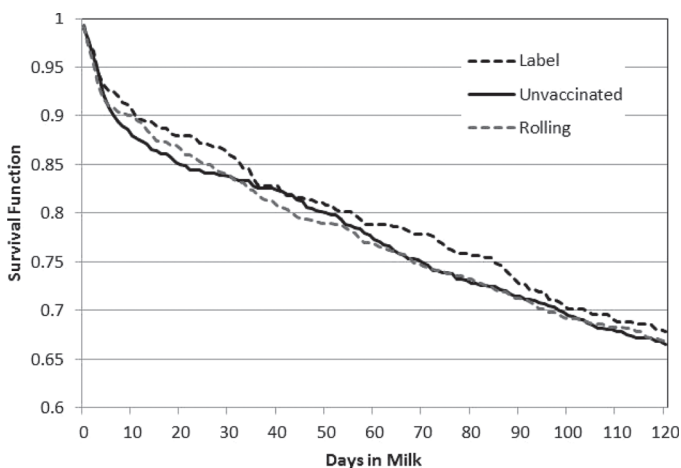


Figure 1. Illustration of the survival of cows to the first case of clinical mastitis in each of the treatment groups. Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were vaccinated at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study. No significant differences were identified between the treatment groups.

Table 5. Summary of individual cow SCC data ($\times 10^3$ cells/mL) from each of the treatment groups in early lactation¹

Item	Unvaccinated group				Label group				Rolling group			
	GM ²	Median	Range	95% CI	GM	Median	Range	95% CI	GM	Median	Range	95% CI
Test-day 1 (5–30 DIM)	97	74	5–9,431	78–116	97	64	4–11,512	73–121	98	74	4–10,514	80–116
Test-day 2 (31–60 DIM)	80	57	3–9,973	68–102	76	53	4–11,995	55–97	76	50	6–9,387	57–95
Test-day 3 (61–90 DIM)	93	64	3–12,512	66–120	88	60	3–8,669	65–111	91	65	5–7,225	72–110
Test-day 4 (91–120 DIM)	96	76	5–9,050	77–115	86	66	4–9,741	69–103	104	83	2–7,799	86–122

¹Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were vaccinated at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study.

²Geometric mean.

groups, with 26.2, 18.3, and 24.2% of cows being culled in the unvaccinated, label, and rolling groups, respectively. Significantly fewer cows were culled from the label group than from either the unvaccinated (76/340 vs. 168/474: $P = 0.02$) or the rolling groups (76/340 vs. 155/483: $P = 0.04$), although the unvaccinated and rolling groups did not differ (168/474 vs. 155/483: $P = 0.88$). Exploration of the data to identify cows in which at least part of the reason for culling was mastitis revealed that 4.2, 3.1, and 3.8% of cows were culled in the unvaccinated, label, and rolling groups, respectively, in the first 305 d of lactation. Although proportionally, the fewest cows were culled for reasons related to mastitis in the label-vaccinated group, the differences were not significant. Multivariable analysis was performed to take into account potentially confounding factors for mastitis-related culling, but failed to identify a significant effect of vaccination.

Milk Production

The effect of vaccination on milk production was assessed using data available from DHI tests. The mean and median individual test-day and total 120-d milk production data of cows in each of the treatment groups are summarized in Table 10 and illustrated in Figure 2. The yields at individual recordings and the calculated cumulative 120-d yields varied significantly between treatment groups ($P < 0.05$). Similarly, total milk protein produced varied significantly between the treatment groups ($P < 0.05$), whereas total milk but-terfat did not; overall milk solids production also varied significantly between treatment groups ($P < 0.01$).

Multivariable analysis demonstrated that, on average, cows in the label group produced a significantly higher volume of milk (231 L; 95% CI: 104.1–357.4) than unvaccinated cows in the first 120 d of lactation. However, when a second model was specified that took into account the occurrence of clinical mastitis and elevated SCC at herd test-days, the impact of vaccination in the label group decreased to approximately 204 L, as summarized in Table 11. As might be expected, we observed significant variation between farms but not between parities. Spring-calving cows yielded between 218 and 516 L more milk than cows calving in other seasons. Culling was associated with the largest reduction in yield (1,892 L; 95% CI: 1,654–1,892). Each 1-L increase in yield at the last recording of the previous lactation was associated with a 17.42-L (95% CI: 11.01–23.83) increase in yield in the first 120 d of the subsequent lactation. A case of clinical mastitis was associated with a reduction in yield of 23.3 L, although this effect was not significant (95% CI: –129.8–83.3); in contrast, each unit log increase in individual cow

Table 6. Summary of the severity of clinical signs of mastitis in cases occurring in the first 120 d of lactation¹

Grade	Unvaccinated group		Label group		Rolling group		Overall	
	n	%	n	%	n	%	n	%
Milk signs only	137	47.24	106	54.92	156	54.36	399	51.82
Milk and udder signs	123	42.41	70	36.27	116	40.42	309	40.13
Systemic signs	22	7.59	12	6.22	12	4.18	46	5.97
Toxic signs	8	2.76	5	2.59	3	1.05	16	2.08
Total cases	290		193		287		770	

¹Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were vaccinated at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study.

SCC at the first and second herd test-days resulted in reductions in yield of 101.8 L (95% CI: 64.9–138.7) and 64.6 L (95% CI: 27.9–101.3), respectively.

A similar model was specified to explore the effect of vaccination on milk solids production, the findings of which are summarized in Table 12. This demonstrated that cows in the label group produced 12.36 kg (95% CI: 3.12–21.60) more milk solids in the first 120 d of lactation compared with unvaccinated herdmates. Again, we observed significant variation between farms and seasons, but, interestingly, not between parities. Culling was associated with the largest reduction in production of milk solids (137.0 kg; 95% CI: 113.5–160.4). A case of clinical mastitis was associated with a reduction in milk solids production of 9.07 kg (95% CI: 0.85–17.29). Each unit log increase in individual cow SCC at the

first and second herd test-days resulted in reductions in milk solids production of 6.87 kg (95% CI: 4.03–9.71) and 4.40 kg (95% CI: 1.58–7.22), respectively.

DISCUSSION

The ultimate target of developing and implementing the use of a vaccine in the control of a disease complex such as mastitis should be displacement of disease; that is, an absolute reduction in the overall incidence of disease, irrespective of cause. However, a possible and plausible outcome is one of substitution rather than displacement; that is, the vaccine effectively controls one opportunistic pathogen such as *E. coli*, but a proportion of the inherently susceptible cows that are now protected from one opportunistic pathogen become

Table 7. Summary of the significant terms in the multilevel logistic regression model^{1,2}

Term	Coefficient	SE	Odds ratio	95% CI	
				Lower	Upper
Label regimen	−0.54	0.26	0.58	0.35	0.98
Rolling regimen	−0.38	0.23	0.69	0.43	1.08
Referent: unvaccinated					
Parity 2	0.92	0.43	2.50	1.06	5.89
Parity 3	0.57	0.41	1.77	0.78	4.04
Parity 4	1.04	0.43	2.82	1.20	6.65
Parity ≥5	0.25	0.39	1.29	0.59	2.81
Referent: Parity 1					
Farm C	4.07	0.61	58.62	17.24	199.34
Farm F	2.95	0.61	19.14	5.62	65.24
Farm H	2.38	0.59	10.85	3.33	35.37
Farm P	2.09	0.62	8.05	2.35	27.61
Farm R	2.95	0.60	19.03	5.70	63.56
Farm T	−1.77	0.91	0.17	0.03	1.05
Referent: Farm B					
Coliform clinical case	0.63	0.19	1.88	1.28	2.77
Referent: Noncoliform case					

¹Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were vaccinated at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study.

²Outcome 1 = a case of severe mastitis in the first 120 d of lactation, 0 = no case of severe mastitis in the first 120 d of lactation. Severe mastitis was defined by more than just changes in the milk (e.g., swollen udder, sick cow).

Table 8. Summary of the significant terms in the multilevel logistic regression model describing the effect of an increasing number of vaccinations on mastitis severity¹

Term	Coefficient	SE	Odds ratio	95% CI	
				Lower	Upper
Number of vaccinations	-0.15	0.06	0.87	0.77	0.98
Parity 2	0.72	0.36	2.06	1.00	4.22
Parity 3	-0.04	0.35	0.96	0.48	1.93
Parity 4	0.08	0.37	1.08	0.51	2.29
Parity ≥5	0.12	0.34	1.13	0.57	2.23
Referent: Parity 1					
Farm C	4.51	0.79	90.56	18.73	437.90
Farm F	3.41	0.80	30.36	6.10	150.96
Farm H	2.99	0.78	19.83	4.15	94.73
Farm P	2.40	0.81	11.00	2.19	55.26
Farm R	3.09	0.79	21.89	4.50	106.48
Farm T	-0.60	0.91	0.55	0.09	3.40
Referent: Farm B					
Coliform mastitis case	0.66	0.23	1.93	1.22	3.04
Staphylococcal mastitis case	0.13	0.26	1.13	0.68	1.90
No growth mastitis case	-0.31	0.46	0.73	0.30	1.83
Referent: other mastitis case					

¹Outcome 1 = a case of severe mastitis in the first 120 d of lactation, 0 = no case of severe mastitis in the first 120 d of lactation.

infected with another (e.g., *Streptococcus uberis*). Although not definitively substantiated, the failure of this study, in common with many other vaccination studies (Hogan et al., 1992; Wilson et al., 2007a; Gurjar et al., 2013), to detect a reduction in the overall rate of clinical mastitis may have been due, in part, to this effect.

Another reason why this and other field studies may have failed to detect any effect of vaccination on the incidence of clinical mastitis may have been that vaccine efficacy in the field is due to a combination of direct and indirect effects. Studies such as this can estimate the direct effect of the vaccine (i.e., efficacy of the use of a vaccine in an individual in which it is used based on a given level of exposure) but cannot easily account for the indirect vaccine effect (i.e., the effect that vaccination of individuals in a population has on the level of exposure by virtue of reduction in the amount of pathogen present in the population; Hal-

loran and Struchiner, 1991). However, given that the primary aim of this study was to look at the efficacy of the vaccine with respect to coliform mastitis control, and that the prevalence of contagious mastitis pathogens in the herds selected was low, we might reasonably expect any indirect effects of the vaccine to be small. This is because of the general assumptions that the primary mode of transmission of coliform mastitis is from the environment to the cow, that infected cows do not make a significant contribution to the level of challenge, and that “contagious” spread of coliforms from cow to cow, although reported (Bradley and Green, 2001), is thought to be relatively rare.

Another recent study (Schukken et al., 2014) has investigated the field efficacy of the polyvalent vaccine described in this paper. In contrast to the current study, that study focused on the efficacy of the staphylococcal component of the vaccine. Their design

Table 9. Summary of culling during lactation in the 3 treatment groups¹

Item	Unvaccinated group		Label group		Rolling group	
	n	%	n	%	n	%
Number of lactations	642		416		638	
All culls in first 120 d in lactation	66	10.3	35	8.4	54	8.5
Mastitis culls in first 120 d in lactation	14	2.2	6	1.4	15	2.4
All culls in first 305 d in lactation	168	26.2 ^b	76	18.3 ^a	155	24.2 ^b
Mastitis culls in first 305 d in lactation	27	4.2	13	3.1	24	3.9

^{a,b}Columns within rows are significantly different ($P < 0.05$).

¹Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were vaccinated at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study.

Table 10. Summary of the effect of vaccination on milk production in the first 120 d of lactation¹

Item	Unvaccinated group			Label group			Rolling group		
	n	Mean	Median	n	Mean	Median	n	Mean	Median
1st test-day yield (L)	601	36.2 ^a	36.8	388	38.3 ^b	38.7	603	37.8 ^{ab}	37.8
2nd test-day yield (L)	576	39.8 ^a	40.3	384	41.7 ^b	42.2	587	41.0 ^{ab}	41.6
3rd test-day yield (L)	561	37.8 ^a	37.9	370	39.8 ^b	39.8	565	38.8 ^{ab}	39.3
4th test-day yield (L)	529	35.1 ^a	35.7	354	37.0 ^b	37.1	546	35.8 ^{ab}	36.4
120-d cumulative yield (L)	644	4,355 ^a	4,532	417	4,619 ^b	4,761	637	4,507 ^{ab}	4,641
1st test-day butterfat (%)	621	4.51	4.40	395	4.51	4.37	623	4.50	4.48
2nd test-day butterfat (%)	594	4.04	4.05	389	4.03	4.00	597	4.06	4.06
3rd test-day butterfat (%)	571	4.11	4.05	375	3.96	3.93	574	4.09	4.06
4th test-day butterfat (%)	531	4.18 ^a	4.12	354	3.94 ^b	4.04	547	4.15 ^{ab}	4.12
120-d cumulative butterfat yield (kg)	621	183.8 ^a	183.7	395	191.4 ^b	191.0	622	189.4 ^{ab}	187.2
1st test-day protein (%)	621	3.23	3.17	395	3.19	3.16	623	3.23	3.17
2nd test-day protein (%)	594	3.02	3.01	389	3.02	3.01	597	3.02	3.01
3rd test-day protein (%)	571	3.11	3.10	375	3.08	3.06	574	3.11	3.10
4th test-day protein (%)	531	3.21	3.18	354	3.17	3.14	547	3.17	3.17
120-d cumulative protein yield (kg)	621	136.6 ^a	141.0	395	144.7 ^b	147.2	623	141.0 ^{ab}	144.0
120-d cumulative solids yield (kg)	621	320.4 ^a	329.0	395	336.1 ^b	341.1	623	330.2 ^{ab}	332.2

^{ab}Columns within rows are significantly different ($P < 0.05$).

¹Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were vaccinated at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study.

took into account both direct and indirect vaccine effects and involved a longitudinal study of subclinical and clinical infections, reporting a reduction in the reproduction ratio for *Staph. aureus* and CNS of 45 and 35%, respectively. Similar findings were unlikely in our study because the focus was on clinical mastitis and environmental rather than contagious mastitis pathogens; furthermore, the incidence of staphylococcal mastitis was much lower in the herds used in this study than in the 2 herds selected by Schukken et al. (2014).

The etiology of clinical mastitis in this study was very complex, with more than 40 genera identified. This level of identification was only possible because of the use of MALDI-TOF MS for bacterial identification. Although this relatively new technique has not been extensively used in mastitis diagnostics, some small-scale studies have demonstrated its utility in this field (Barreiro et al., 2010). In addition, extensive in-house validation, funded by the UK Technology Strategy Board, has been undertaken by the laboratory used in this study (QMMS Ltd.) and the instrument has the IVD-CE mark with a Declaration of Conformity in accordance with the European Commission (1998). Use of this new technique will not have introduced any bias in the study because it was used across all treatment groups. Adoption of high-throughput proteomic techniques such as this are likely to offer an opportunity to refine and enrich our understanding of mastitis etiology, which is still constrained by the limitations of existing techniques, as illustrated by a recent publication reviewing the identification of environmental streptococci (Werner et al., 2014). Moreover, the use of MALDI-TOF MS holds promise as a technique for rapid, cost-effective sub-species strain typing (Rizzardi et al., 2013).

A concern with field studies such as this is the potential for bias. In this study, the risk of bias was minimized by the use of randomization tables in allocation of cows to treatment groups, and because no form of identification was used that allowed easy identification of cows based on their treatment group. Moreover, although bias could have been introduced in the subjective identification of clinical mastitis, other outcomes used in the study such as yield and SCC could not be influenced by study personnel. Additionally, laboratory personnel undertaking somatic cell counting and bacteriology were completely blinded to product administration.

One observation which cause for concern was that, in the analysis, the label group only contained 416 animals, compared to 638 and 642 in the rolling and control groups, respectively. This difference arose from the fact that cows were only included in the analysis if they had received (or would have received) 2 vaccina-

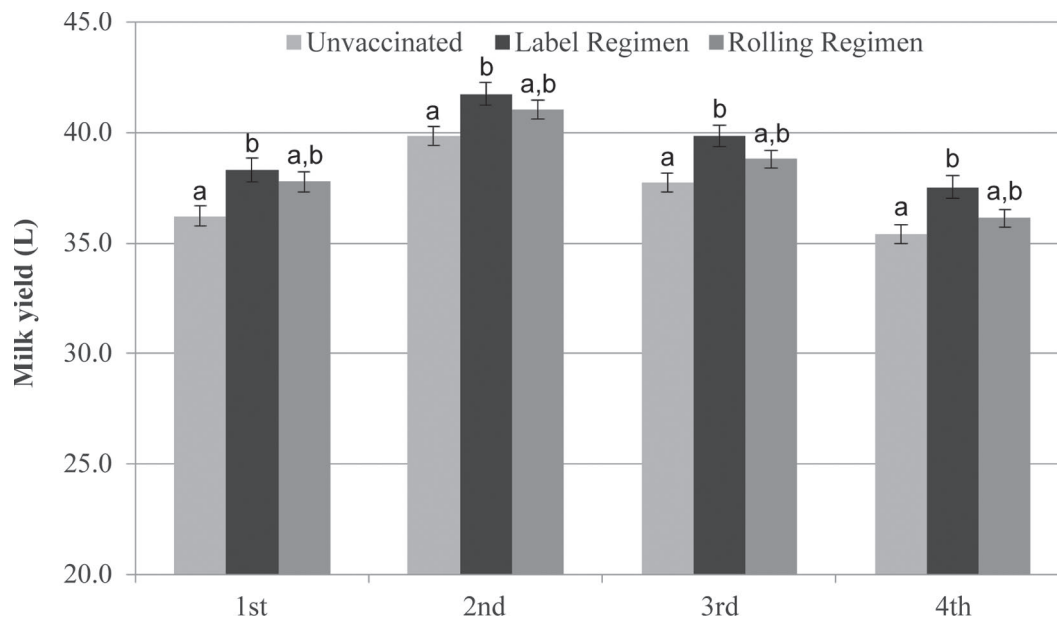


Figure 2. An illustration of the effect of vaccination on milk yield recorded on each of the first 4 DHI test-days (1st to 4th) occurring in the first 120 d of lactation. Cows in the unvaccinated group acted as negative controls and did not receive any vaccinations. Cows recruited to the label group were vaccinated at 45 d before the estimated date of calving (based on herd records), 35 d later, and 52 d postcalving. Cows recruited to the rolling group were vaccinated on the day of recruitment (d 0), 28 d later (d 28), 62 d thereafter (d 90), and then every 90 d until the end of the study. ^{a,b}Different letters within test-day groups are significantly different ($P < 0.05$). Error bars denote SE.

tions by the time of calving. Despite good cow records, achieving this objective was more challenging in the label group than in the rolling group because the label protocol for the vaccine called for precise scheduling of vaccinations around the period of highest perceived risk (i.e., calving and early lactation). This is an important observation because it reflects an important clinical consideration in implementing a mastitis vaccination program—whether the farmer can manage or achieve the required vaccination protocol.

In common with others (Hogan et al., 1992; Wilson et al., 2007a), this study demonstrated that vaccination significantly reduces the severity of clinical disease and that increasing the number of vaccinations is associated with decreasing severity of clinical symptoms. As we might expect, coliform mastitis was associated with more severe disease. Of interest is the very large variation in the odds of severe disease between farms. Several factors are important in determining the severity of coliform mastitis, including the levels of vitamin E and selenium (Smith and Hogan, 1993) as well as negative energy balance (Suriyasathaporn et al., 1999). In practice in the field, it is often assumed that the highest-yielding cows will be those most at risk of negative energy balance and thereby severe disease; in this study, the farm with the highest milk volume (farm T) had the lowest odds of a severe case of mastitis, and the herd with the highest odds of severe disease (farm

C) was the herd with, by far, the highest milk solids production. This effect on severity may also explain, in part, why some studies have reported an apparent reduction in clinical mastitis following vaccination and others have not; it may be that the reduction in the severity of clinical signs on some farms is sufficient to reduce signs below the threshold of detection for unskilled and poorly motivated staff.

Other studies have reported a significant effect of vaccination on culling associated with mastitis (Wilson et al., 2007a), which has been used as the economic justification for the use of J5 vaccines (DeGraves and Fetrow, 1991). In this study, although we observed a significant reduction in the rate of culling in the label group compared with the rolling and unvaccinated groups in the first 305 d of lactation, this was not evident in early lactation during the period of claimed vaccine efficacy. Furthermore, when culling related to mastitis was considered, no significant effects could be identified, which may have been due, in part, to the relatively low proportion of the study herds being culled for mastitis reasons (fertility tended to be the most common reason for culling).

Perhaps the most intriguing and financially important finding of this study is the effect that vaccination had on milk production in early lactation. Earlier studies have demonstrated that vaccination results in less loss in yield in cows experiencing mastitis (Wilson et

Table 11. Summary of the multilevel model exploring the effect of vaccination on cumulative milk yield in the first 120 d of lactation¹

Term	Coefficient	SE	95% CI	
			Lower	Upper
Label regimen	204.23	59.50	85.23	323.24
Rolling regimen	102.37	52.99	-3.61	208.36
Referent: unvaccinated				
Farm C	874.18	95.63	682.92	1,065.44
Farm F	286.73	110.92	64.88	508.57
Farm H	297.45	103.99	89.48	505.42
Farm P	129.87	121.17	-112.47	372.20
Farm R	-131.47	113.20	-357.88	94.94
Farm T	747.77	101.47	544.83	950.70
Referent: Farm B				
Parity 2	-302.41	894.80	-2,092.01	1,487.19
Parity 3	-112.09	895.69	-1,903.48	1,679.30
Parity 4	15.34	895.69	-1,776.05	1,806.73
Parity ≥ 5	54.44	894.68	-1,734.92	1,843.81
Referent: Parity 1				
Summer	-342.85	76.91	-496.66	-189.04
Autumn	-516.66	72.88	-662.43	-370.90
Winter	-218.58	69.15	-356.87	-80.28
Referent: Spring				
LnSCC ² at 1st recording	-101.80	18.44	-138.68	-64.91
LnSCC at 2nd recording	-64.58	18.34	-101.27	-27.89
Animals that were culled	-1,892.05	118.76	-2,129.57	-1,654.52
Animals that developed clinical mastitis	-23.26	53.26	-129.79	83.27
Last recorded yield of the previous lactation (L)	17.42	3.21	11.01	23.83

¹The outcome is a continuous variable cumulative milk yield (L) in the first 120 d of lactation.

²LnSCC = natural log of the SCC.

al., 2007b, 2008), but this is the first study to have demonstrated such an effect in the vaccinated population as a whole. Initial models demonstrated that the label-vaccinated group produced almost 2 L more milk per day in the first 120 d of lactation, whereas cows in the rolling vaccination group produced almost 1 L more per day when compared with the unvaccinated group, although the effect was not significant. What is intriguing in this study is that this effect (albeit diluted) persisted even when one accounts for the effect of a cow being affected by clinical mastitis or having a higher SCC in early lactation. Interestingly, the effect of clinical mastitis was relatively small (only a 23-L reduction in yield) when the effect of increased SCC was accounted for. Although an increase in milk volume is of interest, producers' payments in many markets are linked to the production of milk solids rather than milk volume. Analysis of milk solids production demonstrated a similar effect to that seen with yield, with label-vaccinated cows producing around 12 kg more milk solids in the first 120 d of lactation. The reason for this increase in yield in the vaccinated cows is not immediately apparent, though it would seem fair to assume it was not all due to direct effects of the vaccine on the mammary gland, given that these have been controlled for in the models by inclusion of terms for clinical mastitis and SCC. Further scrutiny of the data

relating to solids production suggests that the effect of vaccination was relatively greater on production of protein than on butterfat; therefore, the butterfat:protein ratio would be lower in vaccinated cows, which could suggest less severe negative energy balance in those cows (Duffield et al., 1997). In this study, all 3 groups of cows were managed together throughout the study period, so it was not possible to determine whether the vaccinated cows produced more milk as a result of higher feed intake or through more efficient feed conversion; this would be an interesting area of further research that might help us better understand this apparently important effect of mastitis vaccination.

We did not attempt to model any economic benefits of vaccination in this study, in part because of the complexity of any production effects. However, we can assume that vaccination is likely to prove economically beneficial in higher-yielding herds experiencing a high proportion of severe mastitis, because even if the increase in milk production is associated with a comparable increase in feed intake, the return is still likely to be significant. In February 2014, the margin over all feed costs reported by DairyCo in the United Kingdom (<http://www.dairyco.org.uk/market-information/farming-data/promar-milkminder-dairy-costings/promar-milkminder-dairy-costings-national/#.U0-BWHJOVaQ>) was 17.88 Pence per liter, suggesting

Table 12. Summary of the multilevel model exploring the effect of vaccination on production of milk solids in the first 120 d of lactation¹

Term	Coefficient	SE	95% CI	
			Lower	Upper
Label regimen	12.36	4.62	3.12	21.60
Rolling regimen	6.21	4.16	-2.11	14.53
Referent: unvaccinated				
Farm C	77.29	7.43	62.43	92.15
Farm F	-0.50	8.59	-17.68	16.68
Farm H	10.17	8.10	-6.03	26.37
Farm P	10.95	9.36	-7.77	29.67
Farm R	2.54	8.80	-15.06	20.14
Farm T	12.86	7.85	-2.84	28.56
Referent: Farm B				
Parity 2	-18.03	68.94	-155.91	119.85
Parity 3	-6.47	68.99	-144.45	131.51
Parity 4	1.39	69.01	-136.63	139.41
Parity ≥ 5	-3.10	68.93	-140.96	134.76
Referent: Parity 1				
Summer	-15.39	5.92	-27.23	-3.55
Autumn	-21.99	5.62	-33.23	-10.75
Winter	-9.52	5.33	-20.18	1.14
Referent: Spring				
LnSCC ² at 1st recording	-6.87	1.42	-9.71	-4.03
LnSCC at 2nd recording	-4.40	1.41	-7.22	-1.58
Animals that were culled	-136.98	11.73	-160.44	-113.52
Animals that developed clinical mastitis	-9.07	4.11	-17.29	-0.85
Last recorded yield of the previous lactation (L)	0.46	0.25	-0.04	0.96

¹The outcome is a continuous variable total milk solids (kg) in the first 120 d of lactation.

²LnSCC = natural log of the SCC.

that the marginal additional yield produced by label-vaccinated cows in this study would have been worth £41.12, offering a return on investment (assuming a vaccine cost of £5.34 per dose) of approximately 2.57:1 with respect to milk yield alone.

CONCLUSIONS

The utility of a commercially available, polyvalent mastitis vaccine was investigated under UK field conditions. Use of the vaccine was not associated with a decrease in the incidence of clinical or subclinical mastitis in the first 120 DIM. However, vaccinated cows were significantly less likely to experience severe clinical mastitis and produced significantly more milk and milk solids than unvaccinated herd mates.

ACKNOWLEDGMENTS

The authors acknowledge the input of the farmers who participated in the research and the financial assistance provided by Hipra UK Ltd. (Nottingham, UK).

REFERENCES

Barreiro, J. R., C. R. Ferreira, G. B. Sanvido, M. Kostrzewa, T. Maier, B. Wegemann, V. Böttcher, M. N. Eberlin, and M. V. dos Santos.

2010. *Short communication*: Identification of subclinical cow mastitis pathogens in milk by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J. Dairy Sci.* 93:5661–5667.
- Bradley, A. J., J. E. Breen, and M. J. Green. 2007a. Mastitis pattern analysis—A fresh look at the analysis of bovine mastitis: Part 1—Somatic cell count data. *UK Vet.* 12:29–35.
- Bradley, A. J., and M. J. Green. 2000. A study of the incidence and significance of intramammary enterobacterial infections acquired during the dry period. *J. Dairy Sci.* 83:1957–1965.
- Bradley, A. J., and M. J. Green. 2001. Adaptation of *Escherichia coli* to the bovine mammary gland. *J. Clin. Microbiol.* 39:1845–1849.
- Bradley, A. J., K. A. Leach, J. E. Breen, L. E. Green, and M. J. Green. 2007b. Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. *Vet. Rec.* 160:253–257.
- Collett, D. 1995. *Modelling Survival Data in Medical Research*. CRC Press Ltd., Boca Raton, FL.
- Darlington, R. B. 1990. *Multiple Tests in Regression and Linear Models*. McGraw-Hill Publishing Company, Singapore.
- DeGraves, F. J., and J. Fetrow. 1991. Partial budget analysis of vaccinating dairy cattle against coliform mastitis with an *Escherichia coli* J5 vaccine. *J. Am. Vet. Med. Assoc.* 199:451–455.
- Duffield, T. F., D. F. Kelton, K. E. Leslie, K. D. Liessemore, and J. H. Lumsden. 1997. Use of test day milk fat and milk protein to detect subclinical ketosis in dairy cattle in Ontario. *Can. Vet. J.* 38:713–718.
- Erskine, R. J., E. J. VanDyk, P. C. Bartlett, J. L. Burton, and M. C. Boyle. 2007. Effect of hyperimmunization with an *Escherichia coli* J5 bacterin in adult lactating dairy cows. *J. Am. Vet. Med. Assoc.* 231:1092–1097.
- European Commission. 1998. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices. Accessed Dec. 8, 2014. <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0079&from=EN>.
- Goldstein, H. 1995. *Multilevel Statistical Models*. 2nd ed. Edward Arnold, London, UK.

- González, R. N., J. S. Cullor, D. E. Jasper, T. B. Farver, R. B. Bushnell, and M. N. Oliver. 1989. Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherichia coli* vaccine. *Can. J. Vet. Res.* 53:301–305.
- Green, M. J., A. J. Bradley, G. F. Medley, and W. J. Browne. 2008. Cow, farm, and herd management factors in the dry period associated with raised somatic cell counts in early lactation. *J. Dairy Sci.* 91:1403–1415.
- Green, M. J., P. R. Burton, L. E. Green, Y. H. Schukken, A. J. Bradley, E. J. Peeler, and G. F. Medley. 2004. The use of Markov chain Monte Carlo for analysis of correlated binary data: Patterns of somatic cells in milk and the risk of clinical mastitis in dairy cows. *Prev. Vet. Med.* 64:157–174.
- Green, M. J., K. A. Leach, J. E. Breen, L. E. Green, and A. J. Bradley. 2007. National intervention study of mastitis control in dairy herds in England and Wales. *Vet. Rec.* 160:287–293.
- Gurjar, A. A., S. Klaessig, S. A. Salmon, R. J. Yancey Jr., and Y. H. Schukken. 2013. Evaluation of an alternative dosing regimen of a J-5 mastitis vaccine against intramammary *Escherichia coli* challenge in nonlactating late-gestation dairy cows. *J. Dairy Sci.* 96:5053–5063.
- Halloran, M. E., and C. J. Struchiner. 1991. Study designs for dependent happenings. *Epidemiology* 2:331–338.
- Hogan, J. S., W. P. Weiss, D. A. Todhunter, K. L. Smith, and P. S. Schoenberger. 1992. Efficacy of an *Escherichia coli* J5 mastitis vaccine in an experimental challenge trial. *J. Dairy Sci.* 75:415–422.
- International Dairy Federation. 1981. Laboratory methods for use in mastitis work. Bulletin no. 132. IDF, Brussels, Belgium.
- International Dairy Federation. 1995. Enumeration of somatic cells. FIL-IDF Standard no. 148A. IDF, Brussels, Belgium.
- National Mastitis Council. 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council Inc., Madison, WI.
- Quinn, P. J., M. E. Carter, B. Markey, and G. R. Carter. 1994. *Clinical Veterinary Microbiology*. Wolfe, London, UK.
- Rasbash, J., W. J. Browne, M. Healy, B. Cameron, and C. Charlton. 2005. MLwiN Version 2.02. Multilevel Models Project, Institute of Education, London, UK.
- Rizzardi, K., T. Wahab, and C. Jernberg. 2013. Rapid subtyping of *Yersinia enterocolitica* by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for diagnostics and surveillance. *J. Clin. Microbiol.* 51:4200–4203.
- Schukken, Y. H., V. Bronzo, C. Locatelli, C. Pollera, N. Rota, A. Casula, F. Testa, L. Scaccabarozzi, R. March, D. Zalduendo, R. Guix, and P. Moroni. 2014. Efficacy of vaccination on *Staphylococcus aureus* and coagulase-negative staphylococci intramammary infection dynamics in 2 dairy herds. *J. Dairy Sci.* 97:5250–5264.
- Smith, K. L., and J. S. Hogan. 1993. Environmental mastitis. *Vet. Clin. North Am. Food Anim. Pract.* 9:489–498.
- Suriyasathaporn, W., A. Daemen, E. N. Noordhuizen-Stassen, S. J. Dieleman, M. Nielen, and Y. H. Schukken. 1999. Beta-hydroxybutyrate levels in peripheral blood and ketone bodies supplemented in culture media affect the in vitro chemotaxis of bovine leukocytes. *Vet. Immunol. Immunopathol.* 68:177–186.
- Werner, B., P. Moroni, G. Gioia, L. Lavín-Alconero, A. Yousaf, M. E. Charter, B. Moslock Carter, J. Bennett, D. V. Nydam, F. Welcome, and Y. H. Schukken. 2014. Short communication: Genotypic and phenotypic identification of environmental streptococci and association of *Lactococcus lactis* ssp. *lactis* with intramammary infections among different dairy farms. *J. Dairy Sci.* 97:6964–6969. <http://dx.doi.org/10.3168/jds.2014-8314>.
- Wilson, D. J., and R. N. González. 2003. Vaccination strategies for reducing clinical severity of coliform mastitis. *Vet. Clin. North Am. Food Anim. Pract.* 19:187–197.
- Wilson, D. J., Y. T. Grohn, G. J. Bennett, R. N. González, Y. H. Schukken, and J. Spatz. 2007a. Comparison of J5 vaccinates and controls for incidence, etiologic agent, clinical severity, and survival in the herd following naturally occurring cases of clinical mastitis. *J. Dairy Sci.* 90:4282–4288.
- Wilson, D. J., Y. T. Grohn, G. J. Bennett, R. N. González, Y. H. Schukken, and J. Spatz. 2008. Milk production change following clinical mastitis and reproductive performance compared among J5 vaccinated and control dairy cattle. *J. Dairy Sci.* 91:3869–3879.
- Wilson, D. J., B. A. Mallard, J. L. Burton, Y. H. Schukken, and Y. T. Grohn. 2007b. Milk and serum B-specific antibody responses, milk production change, and clinical effects following intramammary *Escherichia coli* challenge for J5 vaccinate and control cows. *Clin. Vaccine Immunol.* 14:693–699.
- Wilson, D. J., B. A. Mallard, J. L. Burton, Y. H. Schukken, and Y. T. Grohn. 2009. Association of *Escherichia coli* J5-specific serum antibody responses with clinical mastitis outcome for J5 vaccinate and control dairy cattle. *Clin. Vaccine Immunol.* 16:209–217.