Follow-up of farm use of a vaccine against staphylococcus and coliform mastitis (Startvac ND, Hipra)

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Headline

Vaccination with Startvac, the first vaccine against mastitis to obtain a European MA, was evaluated in a farm follow-up study: rigorous implementation; interesting effects on cells and the number of antibiotic treatments.

Résumé

Un suivi de vaccination avec un vaccin commercial destiné à la prévention des mammites à staphylocoques et à coliformes (Startvac ND, Hipra) a été réalisé par 9 praticiens dans 11 élevages de leurs clientèles dans différentes régions. Les élevages sélectionnés devaient présenter au moins 50 % d'analyses bactériologiques correspondant aux valences du vaccin. L'édition d'un calendrier de vaccination calculé à partir des dates d'insémination fécondante est apparue indispensable pour faciliter l'observance du protocole de vaccination par les éleveurs. Par rapport au lot témoin intra-élevage non vacciné, la vaccination s'est traduite chez les multipares par une réduction de plus de 25% des concentrations cellulaires du lait, soit environ 100 000 cellules/ml en valeur absolue ($P<0,05$), et par près de 30% de traitements antibiotiques en moins pendant la lactation ($P<0,05$).

Summary

Follow-up of vaccination with a commercial vaccine designed to prevent staphylococcal and coliform mastitis (Startvac ND, Hipra) was performed by 9 veterinarians in 11 dairy farms from among their clients in different French dairy regions. In the selected herds, at least 50% of bacteriological isolations corresponded to the activity spectrum of the vaccine. Publication of a vaccination schedule calculated from successful insemination dates appeared to be vital in facilitating compliance with the vaccination protocol by the farmers. Compared to a nonvaccinated intra-herd control group, the vaccination resulted in a reduction of more than 25% of milk somatic cell counts in multiparous cows, i.e. approximately 100,000 fewer cells/ml ($P<0.05$), and in nearly 30% fewer antibiotic treatments during lactation ($P<0.05$).

1- Introduction

The risk of udder infection in dairy cows is highest during the dry period and in the first weeks of lactation. These infections manifest as elevated concentrations of somatic cells in the milk and as clinical mastitis, and lead to use of several curative antibiotic treatments and financial losses.

To date, prevention of udder infections has essentially consisted in reducing exposure of quarters to pathogens, especially by improving milking and housing hygiene, and by applying preventive treatments during drying-off.

However, strengthening the animal's defences against udder infection has up to now played little part in preventive strategies. At the most, measures to prevent impairment of the animal's natural defences have been used: preserving the teat's defences by avoiding injuries during milking and bedding; genetic selection based on milk somatic cell concentrations[9]; maintaining the bactericidal activity of polynuclear neutrophils by providing anti-oxidants such as selenium and vitamin E[6] or through specific zootechnical management techniques during the dry period to reduce the risk of ketosis.[5]

In the next few years, we should see the development of operational methods to increase the resistance of cattle to udder infections.

New methods of genetic improvement based on genomics are particularly promising in the field of disease resistance.[3] Stimulating immunity against udder infections is another investigational line that has been explored for decades,[8] but has yet to culminate in the development of an operational vaccine.

The vaccine under study (Startvac ND, Hipra) is an inactivated vaccine combining two types of antigen: the *Escherichia coli* mutant strain J5 which is already widely used in the United States in vaccines against Gram-negative mastitis, and the SP 140 strain of *Staphylococcus aureus* producing high amounts of the extracellular matrix biofilm. The vaccine is administered as 3 injections during the dry period and the start of lactation. In a clinical efficacy study, it led to a more than 50% reduction in new udder infections due to *Staphylococcus aureus*, coagulase-negative staphylococcus and coliforms in vaccinated cows.[7] These results explain why Startvac has become the first mastitis vaccine, and currently it is the only one to have obtained a European MA.

The follow-up study reported in this article had two objectives:

- to observe the implementation of Startvac vaccination in field conditions: selection of herds to vaccinate; discrepancies between planned and actual vaccination dates; practical aspects of organising the vaccination.

- to evaluate the effects of the vaccination on criteria of interest to the farmer: cells in the milk, clinical mastitis, antibiotic treatment, milk production.

2- Materials and methods

21- Protocol principles

The principles of the study protocol were as follows:

1st) Selection of farms likely to benefit from the Startvac vaccination.

2nd) Randomisation of animals into two groups in each selected farm: to be vaccinated (vaccine group) or not (control group).

3rd) Analysis of discrepancies between the actual dates of vaccine injection and the dates corresponding to the administration schedule reported in the MA.

4th) Evaluation, by comparing the vaccine group with the control group, of the effects of vaccination on milk somatic cell concentrations, frequency of clinical mastitis, number of antibiotic treatments (primary endpoint) and milk production (secondary endpoint).

5th) At the end of the follow-up: opinion of farmers and veterinarians with regard to the implementation of the vaccination and its effects.

22- Selection of farms

Veterinarians from different regions of France were invited to propose farms from among their clientele for the vaccination follow-up study.

A dossier was created for each of these candidate farms and included not only general information about the farm (number, breed, milk production, type of milking system, type of housing, etc.), but also the milk somatic cell concentration and the frequency of clinical mastitis in the last 6 months. It was also recommended to perform bacteriological analyses, ten for an average herd of 60 cows, on quarters affected by subclinical or clinical mastitis, according to problems found in the farm.

The farms selected for the follow-up had to have a confirmed subclinical mastitis problem (at least 20% of milk somatic cell counts greater than 300,000 cells/ml) or clinical mastitis (more than 15% of cows affected by clinical mastitis in the previous 6 months) with at least 50% of bacteriological analyses indicating the presence of bacterial species lying within the spectrum of activity of the vaccine: staphylococcus (coagulase-positive or -negative) and coliforms.

Eleven farms distributed throughout the principal dairy regions of France were monitored by 9 veterinarians (Table 1) between July 2009 and April 2010.

Table 1: Farms and veterinarians participating in the vaccination follow-up study

Code	Farm	Address	Veterinarian
LEPA	Gaec de Kereven	29260 PLOUDANIEL	Philippe LE PAGE
KIMO	Gaec de Monte en Roye	08290 BOSSUS LES ROMIGNY	Pierre KIRSCH
KIBA	Gaec du Bel Air	08260 AUVILLERS LES FORGES	Pierre KIRSCH
GUEN	Gaec du Rossignol	35420 P0ILEY	Jean-Yves GUENA
ESQU	EARL de Banos	40400 BEGAAR	Hélène ESQURIAL
FRAS	Gaec des Roches	39130 SAFFLOZ	Jérôme FRASSON
SATR	Gaec Troullier	15100 SAINT GEORGES	Olivier SALAT
SAPB	Gaec du Plomb	15300 VALUEJOLS	Olivier SALAT
LEIS	Gaec Liboreaux	49120 BEGROLLES EN MAUGES	Etienne LEISEING
MONV	Gaec des Peupliers	49280 LA SEIGUINIERE	Thomas MONVILLE
TRIO	SCL dela Saussaye	14400 MOSLES	Arnaud TRIOMPHE

23- Compared treatments

The experimental treatment consisted of 3 intramuscular injections of Startvac into the neck administered by the farmers. The recommended administration protocol was as follows:

- the first injection, 45 days before the predicted calving date with a deviation of 7 days before or after;

- the second injection, 35 days later (10 days before the predicted calving date), with a deviation of 5 days before and 0 days after. If calving occurred before the date of the second injection, then it was recommended to postpone the second injection to 15 days after calving;

- the third injection, 62 days later (52 days after the predicted calving date) with a deviation of 7 days before or after.

The animals in the control group did not receive placebo.

24- Treatment randomisation

The veterinarian created the two animal groups, to be vaccinated or not, in each farm before the start of the follow-up study.

Nulliparous heifers and cows were randomised separately.

For each category of animals, the computer-assisted procedure was as follows:

- animals sorted in ascending order of predicted calving date, which was calculated from the date of successful insemination added to the average gestational period of the breed (282 days for the Prim'Holstein breed, 287 for the Montbéliarde breed);

- random draw to determine the group of the first animal in the list (vaccine or control);

- successive animals in the list were alternately allocated to the two groups.

This procedure was applied to 9 of the 11 farms in the study. In two farms (SATR and SAPB), animals were allocated alternately to either group according to the working number.

The initial groups formed were not modified as a result of animals withdrawing before the start of vaccination or during follow-up, thereby avoiding rushed re-allocations that could have been a source of errors.

25- Vaccination schedule and optimal periods for injection

The software used to allocate the treatments could also then be used to prepare a vaccination schedule calculated from the predicted dates of calving and showing, for any given day, the number of the animals who should receive a vaccine injection, the number of this injection and the deviation allowed in days before and after the predicted date.

A copy of the vaccination schedule was given to each farmer to help him implement vaccination. The farmer was also required to write the actual dates of vaccination on this schedule.

The pertinence of the actual dates of each vaccine injection was estimated *a posteriori*, by

comparing them to the optimal period. Said period was determined by taking into account the dates calculated using the actual calving dates and by applying the deviations mentioned above.

26- Implementation of vaccination and collection of information

The veterinarian helped the farmers implement the vaccination programme in the following ways:

- supplying Startvac doses and all material required to carry out the vaccine injections;
- providing explanations and a demonstration of injection administration.

During the visits to the farm, the veterinarian also made sure that the vaccine was only administered to animals in the vaccine group and that the farmer recorded clinical mastitis and its treatment.

The following information was collected:

- milk production and somatic cell concentration (SCC) in the milk of each cow during the first 4 controls of the current lactation and during the first 4 controls of the previous lactation (Milk Control data) ;
- detected clinical mastitis and antibiotic treatment applied during the first 4 months of the current lactation and during the first 4 months of the previous lactation. In addition, at the end of follow-up, each farmer and his/her veterinarian completed a questionnaire with their opinion regarding the implementation and effects of the vaccination.

27- Statistical analysis of the milk somatic cell concentration data

Three variables were analysed:

- ***The arithmetic mean of the natural logarithm of the SCC*** of the first 4 controls of the current lactation (LnSCC).

A logarithmic transformation was thus applied to the raw data of the milk somatic cell concentration to obtain a near-normal distribution.

This variable was subjected to an analysis of variance according to the General Linear Model model (GLM) which made it possible:

- to verify, through a covariate, the inter-group variations of SCC in the previous lactation (before the vaccination) when considering the change in SCC from one lactation to the next
- to distinguish the fixed effect of the treatment from the fixed effect of each farm
- to determine, via the treatment-farm interaction test, whether the treatment was homogeneous or not for each farm.

For primiparous cows, there was no covariate for previous lactation.

For the per-farm analyses, there was no category variable related to the farm.

If the treatment-farm interaction was non-significant ($P > 0.05$) in the full model, analysis of variance would be performed on a simplified model without interaction.

- ***The percentage of animals with a mean SCC of the first four controls during***

***lactation greater than the threshold of* 200,000 cells/ml**.

- ***The percentage of animals with at least one SCC greater than the threshold of* 300,000 cells/ml *among the first 4 controls during lactation***.

The statistical significance of the percentage differences between the vaccine group and the control group and also between the previous and current lactations, was analysed using the Chi-square test.

28- Statistical analysis of the clinical mastitis and antibiotic treatment data

Three variables were analyzed:

- the number of cows affected at least once by clinical mastitis during the first 120 days of lactation, divided by the number of cows exposed;

- the number of clinical cases, excluding recurrences at less than 3 weeks in the same quarter, divided by the number of quarters exposed. Clinical recurrences occurring in the same quarter within three weeks were not considered to be a new case of clinical mastitis.

- the number of antibiotic treatments, including treatment of recurrences, divided by the number of quarters exposed. In case of clinical mastitis affecting several quarters, each treatment via the intramammary route was counted as one unit. Every systemic treatment was counted as one unit, regardless of the number of quarters affected in the treated cow.

The statistical significance of the percentage differences between the vaccine group and the control group and also between the previous and current lactations was analysed using the Chi-square test.

29- Analysis of the milk production data

The arithmetic mean of milk production during the first 4 controls of the current lactation was subjected to an analysis of variance according to a GLM model with the same structure and same analysis capacities as the model described in section 27 for milk somatic cell concentrations.

3- Results

31- Characteristics of selected farms

There was a rather significant difference between farms in terms of number of cows, mean milk production and mastitis (Table 2).

Table 2: Number, breed, milk production and extent of mastitis in the followed farms

Farm	No. cows	Breed	Kg milk/cow /year	% SCC < 300,000*	% cows with clinical mastitis*
LEPA	145	Holstein	8500	68%	31%
KIMO	50	Holstein	8300	79%	25%
KIBA	110	Holstein	8400	74%	
GUEN	75	Holstein	7500	< 70%	67%
ESQU	90	Holstein	9500	86%	40%
FRAS	70	Montbéliarde	7500	62%	13%
SATR	150	Montbéliarde	5500	77%	12%
SAPB	75	Holstein	8000	88%	16%
LEIS	70	Holstein	8300	80%	
MONV	50	Holstein	9000	81%	18%
TRIO	185	Holstein	7800	<70%	49%

* during the 6 months prior to inclusion

The cows were milked in a herringbone milking parlour in 8 farms, a side-by-side parlour in 1 farm and in a rotary milking parlour in 2 farms. They slept in cubicles in 9 farms, in a strawed area in 1 farm and in a sawdust area in 1 farm.

Antibiotic treatment during the drying-off period was systematic in 9 farms and selective in 2 farms (LEIS and SAPB). With regard to systematic treatment, only 3 of the farms used the same intramammary preparation on all the animals (KIMO, SATR, TRIO). Systemic treatments were given as additional treatment in some infected cows in 2 farms (KIMO, KIBA). With regard to selective antibiotic treatment, non-treated cows received an internal teat sealant (Orbeseal ND, Pfizer). The teat sealant was given in addition to antibiotic treatment in cows deemed to be at risk in 3 farms (MONV, KIBA, ESQU). The records of treatments applied during the drying-off period, for all farms considered together, did not show a considerable difference between the vaccine group and the control group.

The number of bacteriological analyses undertaken by the veterinarians before inclusion of the farms ranged from 3 to 16 (Table 3); the analysis rate based on the number of cows per being between 10% and 67% according to the farm. Analyses, most commonly performed in the clinic (7 farms) or a specialised laboratory (4 farms), revealed a wide variety of situations: a strong predominance of staphylococci in 6 farms (LEPA, KIBA, FRAS, SATR, LEIS and TRIO), average to high percentage of coliforms in 3 farms (ESQU, SAPB et GUEN), and more balanced profiles with a non-negligible rate of streptococci or enterococci in 2 farms

(KIMO, MONV). Overall, 75% of infections analysed prior to inclusion of the farms corresponded to the activity spectrum of the vaccine.

Table 3: Numbers and results of bacteriological analyses performed prior to the inclusion of farms

Farm	No. analyses	No. cows included	Bacteriological identifications
LEPA	9	41	5 S. aureus, 3 CNS; 1 Str uberis, 2 negatives
KIMO	14	36	5 CNS, 4 Str uberis, 1 S aureus, 1 coliform, 3 negatives
KIBA	16	53	8 CNS, 2 S aureus, 3 Strepto, 1 Enterococcus, 3 negatives
GUEN	12	31	6 S. aureus, 5 Enterobacter, 2 Str uberis, 1 Str agalactiae, 1 CNS, 1 negative
ESQU	9	26	3 Klebsiella, 3 CNS, 1 E. coli, 2 negatives
FRAS	11	43	4 S. aureus, 3 CNS, 1 Str dysgalactiae, 1 A. pyogenes, 1 negative
SATR	9	68	6 S. aureus, 2 Str dysgalactiae, 1 Str uberis
SAPB	3	30	2 E. coli, 1 CNS
LEIS	11	31	6 CNS, 4S aureus, 1 negative
MONV	7	36	4 E. coli; 3 Enterococcus sp; 2 Str uberis; 1 S aureus, 1 CNS, 1 negative
TRIO	10	15	4 S. aureus; 3 CNS, 2 streptococci, 1 Gram-negative, 1 negative

32- Timing of vaccine injections in relation to optimal periods

Results in table 4 show that a considerable proportion of the vaccine injections were performed outside of the optimal period, especially for the second injection.

The differences can be partly explained by the difference between the actual calving dates, used to calculate the optimal periods, and the predicted calving dates, used to establish the vaccination schedule. Only 77% of the actual calving dates fell within the interval running from 7 days before to 7 days after the predicted calving date, with 11% falling before and 11% after. The actual versus predicted date differences accounted for about half of the deviations observed for the first vaccine administration (19% before, 22% after) and 80% of deviations for the 3rd injection (14% before and 11% after), which the farmer could time better because it occurred after calving.

The highest deviation was observed for the second injection with more than 70% occurring after the optimal period. This finding can be explained by the absence of an acceptable deviation in the interval after the predicated date and by the fact that calving sometimes occurred before the date calculated for the second injection.

Table 4: Timing of 3 vaccine injections in relation to optimal periods

Vaccine injections	Timing of vaccine injection in relation to optimal periods			
	Before No. (%)	Optimal period No. (%)	After No. (%)	
1st injection	33 (19%)	105 (62%)	39 (22%)	
2nd injection	16 (9%)	34 (19%)	91* (51%)	36** (20%)
3rd injection	24 (14%)	133 (75%)	20 (11%)	

* from 10 days before to 15 days after the actual calving date

** more than 15 days after the actual calving date

33- Effects of the vaccination on the SCC of multiparous cows

The analysis of variance of LnSCC (arithmetic mean of SCC logarithms of the first 4 controls of the current lactation) using the full model showed no significant interaction between treatment groups (vaccination or control) and farms (P=0.79). Analysis could therefore proceed to the simplified model without interaction.

The results of this analysis showed that the individual somatic cell concentrations in cows during the current lactation are significantly influenced by those of the previous lactation (P<0.0001), by the farm to which the cow belongs (P=0.008), and by the treatment, vaccination or not, that the animal received (P=0.012).

Taking all farms together, the current lactation SCC of the vaccinated cows was 25% (geometric mean) to 30% (arithmetic mean) lower compared with that of the control group (Table 5). In absolute values, the arithmetic mean SCC was approximately 100,000 cells/ml less in the vaccinated cows than in the control cows.

Considering the change in the SCC from the previous to the current lactation, the value is almost constant (–8000 cells/ml) in the vaccinated cows despite an increase in their lactation number versus an increase of 100,000 cells/ml in non-vaccinated cows.

Per-farm results show that the current lactation SCC is lower in vaccinated cows than in control cows in 8 of the 11 farms, and the difference reaches 5% statistical significance in 1 farm (KIBA). In the 3 farms (GUEN, LEIS, MONV) where the current lactation SCC in vaccinated cows was (non-significantly) higher than in control cows, it seems that this was already the case during the previous lactation, before vaccination, with a greater sampling variability in farms with fewer animals.

Table 5: Effect of vaccination on SCC of multiparous cows, all-farm and per-farm

Farm	No. of cows		**Geometric mean and *arithmetic mean* of SCC**				**P***
			Previous lactation		Current lactation		
	Vaccine	Control	Vaccine	Control	Vaccine	Control	
LEPA	19	19	56 *311*	99 *273*	66 *605*	153 *849*	0.31
KIMO	9	9	76 *138*	107 *490*	64 *138*	90 *366*	0.82
KIBA	21	20	81 *188*	60 *141*	35 *67*	61 *219*	0.03
GUEN	14	11	87 *280*	52 *77*	67 *210*	61 *160*	0.81
ESQU	9	6	118 *65*	45 *56*	62 *(237)*	*77* *131*	0.47
FRAS	12	12	107 *372*	90 *402*	73 *179*	78 *171*	0.70
SATR	32	28	44 *190*	63 *210*	46 *119*	68 *229*	0.29
SAPB	12	11	40 *199*	56 *134*	38 *(50)*	75 *217*	0.87
LEIS	12	10	95 *270*	67 *94*	171 *464*	106 *189*	0.35
MONV	8	11	89 *121*	51 *81*	107 *624*	90 *255*	0.98
TRIO	6	9	115 *228*	143 *517*	93 *178*	107 *458*	0.92
TOTAL	154	146	67 *225*	71 *214*	61 *217*	83 *315*	0.012

* Analysis of variance after logarithmic transformation of data using the GLM model (previously described) with the SCC of the previous lactation as a covariate

The percentage of multiparous cows in which the arithmetic mean of the current lactation SCCs was greater than the threshold of 200,000 cells/ml (Table 6) was significantly lower (P = 0.004) in the vaccinated group (21%) than in the control group (37%). Here again, the change in milk somatic cell concentration from one lactation to the next was very slightly favourable in vaccinated cows, but was clearly unfavourable (P=0.06) in non-vaccinated cows.

Table 6: Distribution of cows by the mean SCC of their first 4 controls with a threshold of 200,000 cells/ml, by group (vaccine/control) and lactation (current /previous)

Lactation	Vaccine group		Control group		P***
	*SCC < 200** *No. (%)*	*SCC > 200*** *No. (%)*	*SCC < 200** *No. (%)*	*SCC > 200*** *No. (%)*	
Current	121 (79%)	33 (21%)	92 (63%)	54 (37%)	0,004
Previous	118 (77%)	36 (23%)	108 (74%)	38 (26%)	0,69
P***	0.78		0.06		

* Cows for which arithmetic mean of the SCC of the first 4 controls was lower than 200,000 cells/ml
** Cows for which arithmetic mean of the SCC of the first 4 controls was higher than 200,000 cells/ml
*** Probability associated with the Chi-square test

The significant reduction in SCC of the multiparous cows following Startvac vaccination is also confirmed by the finding of at least one SCC greater than 300,000 cells in the first 4 lactation controls (Table 7). The percentage of cows exceeding this threshold was significantly lower in the vaccinated cows than in their non-vaccinated peers (p=0.002) and remained unchanged from one lactation to the next for the vaccinated group, but significantly increased (P = 0.04) with the increase in parity in the control group.

Table 7: Distribution of cows having at least one SCC greater than the threshold of 300,000 cells/ml, by group (vaccine/control) and lactation (current /previous)

Lactation	Vaccine group		Control group		P***
	*All SCC < 300** *No. (%)*	*At least 1 > 300*** *No. (%)*	*All SCC < 300** *No. (%)*	*At least 1 > 300*** *No. (%)*	
Current	116 (75%)	38 (25%)	84 (58%)	62 (42%)	0,002
Previous	115 (75%)	39 (25%)	102 (70%)	44 (30%)	0.42
P***	1.00		0.04		

* All SCC lower than 300,000 cells/ml during the first 4 lactation controls
** At least one SCC higher than 300,000 cells/ml during the first 4 lactation controls
*** Probability associated with the Chi-square test

34- Effects of the vaccination on the frequency of clinical mastitis and antibiotic treatments in multiparous cows

Table 8 shows that the percentage of cows which had clinical mastitis in the first 120 days of the previous lactation was similar in the vaccinated group and in the control group (P = 0.99). During the previous lactation, the number of affected cows was similar in the 2 groups (P=0.73) but to a significantly lesser extent.

Table 8: Distribution of cows having had at least 1 case of clinical mastitis in the first 120 days of lactation, by group (vaccine/control) and lactation (current/previous)

Lactation	Vaccine group		Control group		P*
	No clinical cases No. (%)	*Clincial cases No. (%)*	*No clinical cases No. (%)*	*Clinical cases No. (%)*	
Current	117 (76%)	37 (24%)	112 (77%)	34 (23%)	0.99
Previous	136 (88%)	18 (12%)	126 (86%)	20 (14%)	0.73
P*	0.01		0.05		

* Probability associated with the Chi-square test

The frequency of clinical cases observed in different quarters of the same cow or the same quarter more than 3 weeks later (thus excluding recurrences) was 25% lower in the vaccine group (7.5%) than in the control group (10%) during the first 120 days of the previous lactation (Table 9); however, this difference is not statistically significant (P=0.13).

Table 9: Clinical cases (excluding recurrences) in the vaccinated group and the control group

Lactation	Vaccine group		Control group		P*
	No. quarters	*No. cases (%)*	*No. quarters*	*No. cases (%)*	
Current	616	46 (7%)	584	59 (10%)	0.13
Previous	616	25 (4%)	584	29 (5%)	0.54
P*	0.01		0.001		

* Probability associated with the Chi-square test

Finally, the total number of antibiotic treatments during lactation, including treatments of recurrences, was 29% lower (P = 0.02) in the vaccinated cows than in the control cows (Table 10).

Table 10: Total number of antibiotic treatments during lactation in vaccinated cows and control group cows

Lactation	Vaccine group		Control group		**P***
	No. quarters	*No. treatments (%)*	*No. quarters*	*No. treatments (%)*	
Current	616	76 (12%)	584	101 (17%)	0.02
Previous	616	40 (6%)	584	41 (7%)	0.80
P*	0.001		<0.0001		

* Probability associated with the Chi-square test

35- Effects of vaccination on the milk production of multiparous cows

The milk production means during the first 4 lactation controls were not significantly different (P=0.59) between the cows in the control group and those in the vaccine group (Table 11).

Table 11: Comparison of SCC of cows in the vaccine group and cows in the control group, during the current lactation and during the previous lactation

Lactation	Vaccine group			Control group			P**
	No. cows	*Milk per cow per day**	*Standard deviation*	*No. cows*	*Milk per cow per day**	*Standard deviation*	
Current	148	31.6	11.2	137	29.7	11.5	0.59
Previous	148	26.3	9.4	137	25.6	10.0	

* arithmetic mean in kg of the first 4 lactation controls

** Analysis of variance using the GLM model previously described with milk production in the previous lactation as the covariate

36- Results obtained in primiparous cows

The results obtained with primiparous cows are summarised in Table 12. Among the analysed criteria, few differences were observed: slightly more cells and slightly fewer clinical mastitis cases in the primiparous cows of the vaccinated group than in those of the control group. However, the differences, derived from low numbers of animals, are far from the statistical significance threshold.

Table 12: Summary of results obtained in primiparous cows

Criterion	Vaccine group (56 cows)		Control group (54 cows)		P*
	Mean	*%*	*Mean*	*%*	
SCC geometric mean in cells/ml	67		59		0.44
SCC arithmetic mean in cells/ml	238000		211000		0.44
% cows with mean SCC > 200,000		27%		20%	0.50
% cows with at least one SCC > 300,000		29%		22%	0.51
% cows with at least one clinical case		13%		19%	0.44
No. clinical cases/100 quarters		3.1%		5.6%	0.31
No. treatments/100 quarters		5.8%		7.4%	0.24
Kg milk/cow/day	25.3		27.0		0.30

*Analysis of variance using GLM model for continuous variables; Chi-square test for percentages

37- Input from farmers and veterinarians

Nine (9) out of 10 farmers found implementation of the vaccination in their farm to be easy or rather easy; only access to the animal to perform the second injection presented some difficulties. For the veterinarians, implementation was judged as being easy or rather easy, with their principal difficulty being the performance of bacteriological analyses for the selection of herds to vaccinate. Both the farmers and the veterinarians considered that the creating a vaccination schedule was vital.

With regard to the effects of the vaccination, the main improvements according to the farmers were clinical mastitis: 7 farmers out of 10 reported a reduction in clinical mastitis cases, in their severity, the number of recurrences and the number of antibiotic treatments applied; the other 3 farmers did not note any change.

With regard to milk somatic cell concentration, 3 farmers felt that there was a slight reduction, while the others did not notice any difference.

5 Discussion

Before undertaking a vaccination programme, it is advisable to check that the dominant udder infections in the farms correspond to the spectrum of activity of the vaccine. It was therefore recommended that a bacteriological survey be conducted and that the vaccination only be implemented in farms in which at least half of the diagnosed infections were due to the species included in the spectrum of activity of Startvac. In practice, veterinarians found this recommendation to be the main obstacle for the implementation of vaccination and the number of analyses varied widely from farm to farm.

These observations underline the need to objectively define, using a statistical approach, the size of the bacteriological survey to conduct[2] and, using a bio-economic simulation, the minimum prevalence threshold of target-species to be used when selecting farms to vaccinate. This information would be very valuable in allowing veterinarians to prescribe vaccination in a well-founded manner.

The vaccination follow-up also revealed occasionally large differences between the actual dates of vaccination and the dates calculated using the protocol in the vaccine's SmPC. The deviations for the first and third injections, which both allowed a deviation of 7 days before and 7 days after, appear to be moderate. These deviations can be largely explained by the difference between the actual and predicted calving date. The difference for the third (post-calving) injection can be explained by the fact that the vaccination schedule was fully drawn up before the start of the follow-up, and was based on the predicted calving date. Calculation of the date of the third injection according to the real calving date would reduce this deviation and should therefore be recommended.

The primary difficulty concerns the second injection, which was planned to be carried out 10 days before the predicted calving date. The decision to have zero deviation for second injections carried out after the predicted calving date seems to have been unrealistic, and

resulted in a considerable percentage of injections being carried out outside of the optimal period. Considering that the second injection must be done 10 days before calving at the latest, then the date calculated based on the vaccination schedule should be pushed forward, for example by 15 days before the predicted calving date, such that a deviation period appears after the indicated date (5 days in our example).

The experimental design of this follow-up study was conceived to evaluate the effects of the vaccine by comparison with an intra-herd control group of non-vaccinated cows. Furthermore, as animals were followed-up during two successive lactations, it was possible to not only analyse the vaccination effects on current lactation, but also to observe changes from the previous lactation (before vaccination) with regard to the current lactation for both groups of cows, whether vaccinated or not. The experimental design made it possible to apply a statistical analysis model that could control background noise (notably related to lactation, year and farm) and thus to increase the power of the study.[4]

This experimental design also makes it possible to reduce, without eliminating, the risks of bias, and to partially control sampling variation. One of the potential biases recalled is the mastitis treatments given during the drying-off period or during lactation. It was seen that these treatments varied greatly between farms and sometimes in the same farm, from one animal or case to the other, sometimes according to a difficult-to-follow logic. Nevertheless, there is nothing to suggest that the farmers changed their treatment practices according to whether the animal belonged to the vaccinated group or the control group – a required condition for this bias. The sampling variation that remains could influence results at the level of the farm, all the more so if numbers are low, but the incidence is negligible when one considers that more than 100 animals, vaccinated or not, were included from all farms combined.[4]

Vaccination with Startvac, in the conditions we have just described, led to a statistically significant decrease (P=0.012) of 25% in the geometric mean (61,000 *versus* 83,000 cells/ml) and of 30% in the arithmetic mean (217,000 *versus* 315,000 cells/ml) of the somatic cell concentration of milk in multiparous cows during the first 4 months of the current lactation. The SCC expressed as a geometric mean offers the advantage of being less sensitive to the influence of the highest values; however, the arithmetic mean is much closer to the values observed in the herd milk and its significance in terms of infection prevalence and economic loss at the level of the herd is demonstrated.[1]

The significant reduction in the milk somatic cell concentration in vaccinated multiparous cows as compared to the control group is also reflected in the two other criteria using cut-off points: % of cows with an SCC mean > 200,000 cells/ml (P = 0.004) or at least one SCC > 300,000 cells/ml (P=0.002).

If we now compare the SCC values in the previous and current lactation, it seems that, regardless of which follow-up criterion is used, there is practically no change in vaccinated cows, while there is a considerable rise in SCC in non-vaccinated cows, primarily due to increasing lactation number.[10] It is as if the protection conferred by the vaccination compensates for the age-related deterioration in udder health.

The absence of a significant interaction (P=0.79) between the treatments (vaccine or not) and farms in the GLM model means that the effect of vaccination on SCC did not vary

significantly between farms. The low number of animals included in each of the farms did not make it possible to demonstrate a statistically significant difference between groups at the farm level, except for one of the farms.

The 29% decrease in antibiotic treatments administered during lactation to the multiparous cows (P= 0.02) combined with the fact that the percentage of cows affected by clinical mastitis at least once was almost identical in the two groups, suggesting that the frequency of recurrences, whether new infections or relapses, was lower in the vaccinated cows. There is also the possibility, which could not be verified in this study, that mastitis cases were more severe in the non-vaccinated group, requiring more treatments. Among the farmers, there was a more distinct perception of the effect of vaccination on clinical mastitis and number of administered antibiotic treatments than on the milk somatic cell concentrations.

The effects of vaccination on the udder state of health of multiparous cows are consistent with the results of the clinical efficacy trial contained in the Startvac MA dossier.[7] This trial reported a reduction of more than 50% in new infections due to staphylococci and coliforms, as well as a rise in the rate of spontaneous resolution in vaccinated cows, reflected in the positive effects on milk somatic cell concentration, clinical mastitis and number of antibiotic treatments administered.

One could reasonably ask whether the differences between the vaccinated and non-vaccinated multiparous cows observed during the first 4 months of lactation are maintained throughout the entire lactation period. Firstly, a few more quarters of the vaccinated group could become infected in the second part of lactation when the vaccine protection has ceased. Moreover, the quarters of the control group that were infected towards the end of the 4-month observational period will express elevated SCC or clinical mastitis mainly after the 4th month of lactation. Finally, as the vaccination was only given to some of the animals, its effects, associated with a decreased contagion and lower pathogen exposure in all the animals, are undoubtedly underestimated.

The lack of a statistically significant difference in milk production between the vaccinated and nonvaccinated multiparous cows was not surprising if one considers that the increase in milk production associated with a 100,000 cells/ml reduction in milk somatic cell concentration has been estimated to be approximately 2%,[1] i.e., a difference of less than 1 kg of milk per day at the start of lactation, which, to demonstrate the difference, would require performing production controls a lot more frequently than the once-monthly Milk Control, especially during the rising phase of lactation.[4]

Moreover, this follow-up study was not able to demonstrate any significant effects of the vaccine on primiparous cows regardless of the follow-up endpoint. The MA study had showed a significant reduction in udder infections in primiparous cows, albeit less marked than in multiparous cows.[7] The study's power was a lot lower for the primiparous cows than for the multiparous cows due to the low number of primiparous cows included in the follow-up study and also the fact that we cannot enter data from previous lactation as a covariate in the analysis model.

It is also worth highlighting that the results reported for the present follow-up study include all udder infections in the farms, including streptococcus infections outside of the spectrum of activity of Startvac, while the MA study results only included staphylococcus and coliform

infections targeted by the vaccine.

It would be interesting to perform a bio-economic simulation taking into account the reduction in the contagion in order to determine the economic interest of the Startvac vaccination in different epidemiological contexts.

6- Conclusion

In view of the results of the follow-up, it seems to have been relatively pertinent to select farms for vaccination on the basis of a bacteriological survey showing that at least 50% of udder infections are due to bacterial species targeted by the vaccine. However, the number of analyses to perform for this survey and the target-species prevalence threshold to be taken into account in order to use the vaccination should be determined based on statistical and bioeconomic studies, and should be included in the vaccine's instructions for use.

A vaccination schedule is an absolute requirement in order for farmers to implement vaccination. Taking into account the actual calving date to calculate the 3rd injection date and having sufficient deviation periods around the calculated calving dates would contribute to reducing the intervals between the actual dates the farmers perform the vaccine injection and the dates planned according to the MA vaccination.

The effects of Startvac vaccination in the followed-up farms were as follows:

- a reduction in the milk somatic cell concentration of multiparous cows of more than 25% in relative terms and approximately 100,000 cells/ml in terms of arithmetic mean ($P = 0.012$) ;

- a reduction of nearly 30% in the total number of antibiotic treatments against mastitis during lactation in multiparous cows ($P = 0.02$).

Farmers had a higher perception of the effects of the vaccine on clinical mastitis and on the number of antibiotic treatments in lactation than on the somatic cell concentration of milk.

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