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RESEARCH ARTICLE

Field Evaluation of *Staphylococcus aureus* Bacterin Use in Dairy Farms

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Abstract

A total of 420 lactating Holstein cows in three dairy farms in Damietta (farm A, 120 lactating cows and farm C, 160 lactating cows) and El-Sharkia (farm B, 140 lactating cows) Governorates; were examined for the presence of clinical and subclinical mastitis among the resident cows, then were vaccinated by *Staph. aureus* bacterin and then were observed during the period from May 2015 to December 2017 for evaluating the efficacy of the vaccine in reducing the number of infected animals and preventing new infections. Clinical mastitis and subclinical mastitis were respectively reported in 286 (17.54%) and 726 (44.53%) out of the total examined quarters (n=1630) from 420 animals in 3 farms. Before vaccination, the overall prevalence rate of *Staph. aureus* was (19.5% versus 46.5%) in mastitic quarters and (32.1% versus 44.6%)in subclinical mastitis. Vaccination of cows with 5 ml of *Staph. aureus* bacterin 2 doses with 14-day interval decrease the severity of mastitis in the new cases of clinical mastitis and reduce the prevalence of *Staph. aureus* and coagulase-negative staphylococcal mastitis, improved clearance rates of existing *Staph. aureus* mastitis, but it has little effect on reducing new mastitic cases**.** However, it was found to be effective in preventing new infections with *Staph. aureus* and coagulase-negative staphylococci in dairy heifers, and minimizing somatic cell count, and increasing milk yield. Also, the total bacterial count, total Staphylococcal count, and *Staph. aureus* count was reduced. Vaccine efficacy was stretched to fat, protein percent and milk yield were elevated.

Keywords: *Staph. aureus*, bacterin, clinical and subclinical mastitis, dairy cow.

Introduction

Mastitis is one of the most ubiquitous diseases of the dairy industry which affect in negative way on the animal production by decreasing the quantity of cow milk and the losses are raised due to exclusion of bad quality milk, culling of infected cows and treatment costs [1, 2]. Mastitis in both forms (clinical and sub-clinical forms) is disappointing, which results in a reduction in both quantity and quality of milk [3]. But the most dangerous type of mastitis to the stockholders is the subclinical mastitis not only it is the most prevalent type, as it is 15 to 40 times more than the clinical mastitis, but also has long duration effect, difficult to be detected, adversely affect components of milk, and is considered a reservoir of microorganisms that can be transmitted to other animals within the farm due to the contagious nature of the disease [4].

Elevation in somatic cell count (SCC) of the milk with isolation of the causative microorganism are the main indicator for the presence of subclinical mastitis. Most recent research seems to agree to a cut-off point at about 250,000 cells/ml, milk production decreases linearly with an increase in SCC. Decreased milk production in combination with the costs of treatment and culling due to mastitis form the major costs of mastitis. Mainly, subclinical mastitis is caused by *S. aureus* and *Strept. agalactiae* and a few of other Streptococci [5, 6]. *Staph. aureus* mastitis is the most important infectious disease that affects both the quantity and quality of milk manufacture. Intramammary antibiotic therapies formulated for the treatment of mastitis are generally unsuccessful in eliminating existing *Staph. aureus* mastitis. Despite applying intensive control measures, it is greatly difficult to eliminate *Staph. aureus* mastitis and remains a serious economic problem for dairy industries [7,4]. Vaccination is a reasonable approach to the control of *S. aureus* mastitis. However, the available *S. aureus* vaccine has shown limited efficacy under field conditions, mainly due to the lack of information concerning relevant antigens which will produce a broad-spectrum immunization, thus the development of effective methods of controlling *S. aureus* mastitis is necessary which leads to reduced costs and increase dairy productivity. Vaccination against *S. aureus* mastitis appears to be the logical method to control the disease but need more researches [8].

Aim of work was directed mainly to study the prevalence rates of clinical and sub-clinical mastitis in lactating dairy cows, demonstrating the prevalence of Staphylococcal infections in clinical and subclinical cases, and evaluating Staphylococcal bacterin in reducing the prevalence of mastitis and lowering somatic cell counts.

Material and Methods

Animals

A total of 420 lactating Holstein cows in three dairy farms in Damietta (farm A, 120 lactating cows and farm C, 160 lactating cows) and El-Sharkia (farm B, 140 lactating cows) Governorates at different lactation stages, different lactation seasons, and under different hygienic measures.

Experimental protocol

Cows under experiment were examined for clinical and subclinical mastitis then were vaccinated by *Staph. aureus* bacterin. Observation and follow up of the vaccinated cows during the period from May 2015 to December 2017 to evaluate the reduction rate of infection and the rate of production after vaccination. Milk production per cow and the bulk tank analysis were assessed (total bacterial counts, total Staphylococcal counts, SCC, milk fat, and milk protein). Also, hygienic measures, management, milking process, disinfectant, and dry cow therapy were studied concerning mastitis.

Samples

Milk samples:

A total of 1916 quarter milk samples from clinical and subclinical mastitic quarters out of 1930 quarters examined were collected for bacteriological examination before and after vaccination from the three farms as following:

A total of 286 and 726 quarter milk samples from clinical and subclinical mastitic quarters before vaccination, respectively (at ZERO days). A total of 192 and 490 quarter milk samples from clinical and subclinical mastitic quarters after vaccination, respectively. Vaccinated lactating cows in the three dairy farms were examined every 2 weeks for 120 days after the administration of the second dose of the bacterin for clinical and sub-clinical mastitis by clinical examination and by using California mastitis test (CMT) for detection of subclinical mastitis. A total of

55 and 167 quarter milk samples from new clinical and subclinical mastitic quarters appeared respectively during 120 days of lactation after vaccination were subjected to clinical examination and by using CMT for detection of subclinical mastitis every 2 weeks.

A total of 15 bulk tank milk samples from farm milk tank from each farm for bulk tank analysis (first sample before vaccination at zero-day, then milk samples from the tank every month after vaccination for 4 successive months to each farm) were taken to study the effect of vaccination on total bacterial counts, total Staphylococcal counts, *Staph. aureus* counts, SCC, milk fat, and milk protein of bulk tank milk were assessed after administration of booster dose of the bacterin monthly for 4 months [9].

California mastitis test were done according to Radostitis *et al.*, [1] for detection of sub-clinical mastitis.

Isolation and identification of Staphylococcus species Culture procedures were done as described by the National Mastitis Council [9].

Vaccine and vaccination procedure (*Staph. aureus* Bacterin), **Lysigin®** [10], is a lysed culture of highly antigenic polyvalent somatic antigen containing phage types I, II, III, IV, and miscellaneous groups of *S.* aureus combined with an aluminum hydroxide adjuvant and includes capsular serotypes 5, 8, 336. Boehringer Ingelheim, Inc. St. Joseph, MO 64506 U.S.A. U.S. Veterinary License No. 124.

To conduct the analysis, all lactating Holstein cows in the three dairy farms (at different lactation stages, different lactation seasons, and under different hygienic measures) were vaccinated with *Staph. aureus* bacterin (Lysigin®) according to manufacturer's instructions using a 5-mL dose that was injected intramuscularly in the gluteal muscle with a booster dose 14 days later. Efficacy of vaccination was observed during the 120 days of lactation. This period was selected because it corresponded to the period of expected efficacy when following the label vaccination regimen. The effect of vaccination on clinical mastitis, subclinical mastitis, total bacterial counts, total staphylococcal counts, *S. aureus counts*, somatic cell counts, milk fat and protein of bulk tank milk, milk yield average per cow, and culling rates per farm were assessed and compared with the data of the survey done before vaccination.

Hygienic measures, milking process, management, and dry cow therapy

Cows from the three farms were investigated under different hygienic measures, milking process, management, and dry cow therapy as shown in Table (1).

Effect of vaccination with Staph. aureus bacterin on clinical and subclinical mastitis

Vaccinated lactating cows in the three dairy farms were examined every 2 weeks for 4 months after the administration of the second dose of the bacterin for clinical and subclinical mastitis. The collected mastitic milk samples of clinical and sub-clinical mastitis occurring during the 120 days of vaccination were refrigerated at 4°C and sent to the laboratory; samples were processed within 4 to 8 hrs of sampling. The microbiological assays and diagnosis of mastitis were carried out as indicated previously for the presence of staphylococci species and matched with the data of clinical and sub-clinical mastitis before herd vaccination.

Effect of vaccination with Staph. aureus bacterin on total bacterial counts, total Staphylococcal counts, Staph. aureus counts, somatic cell counts, milk fat and milk protein of bulk tank milk

The effect of vaccination on total bacterial counts, total Staphylococcal counts, *Staph. aureus* counts, SCC, milk fat, and milk protein of bulk tank milk was assessed after administration of booster dose of bacterin monthly for 4 months. About 40 ml of bulk tank milk of vaccinated cows were taken aseptically every month in a sterilized plastic tube, then cooled and transported to the laboratory for the investigation. The microbiological assays were carried out as indicated previously. The Bulk tank milk analysis in vaccinated cows from the dairy herds was matched by bulk tank milk before vaccination.

Effect of vaccination with Staph. aureus bacterin on milk yield average per cow

The effect of vaccination on milk yield was assessed during the 120 days of vaccination. Kilograms of milk production was recorded in vaccinates daily and the average of milk production was calculated and compared with the milk yield average before vaccination.

Effect of vaccination with Staph. aureus bacterin on dairy cow culling rates per farm in different dairy farms

The overall mastitis-specific culling rates were calculated during 120 of vaccination and 305 days of lactation and compared with donated data of the farm records.

Effect of vaccination with Staph. aureus bacterin on the frequency of new Staph. aureus IMI

A total of 618 lactating quarters free of any clinical or sub-clinical signs were examined for clinical and sub-clinical mastitis every 2 weeks for 120 days of lactation after vaccination as previously mentioned. Samples were processed within 4-8 hrs of sampling. The microbiological assays and diagnosis of mastitis were carried out as indicated previously.

Results

The prevalence rate of clinical and subclinical mastitis

The overall prevalence rate of mastitis in the different studied dairy farms under unlike hygienic measures among 420 examined lactating cows at the level of quarters was 62.08% (1012/1630), of which 17.54% (286/1630) and 44.53% (726/1630) were clinical and subclinical mastitis, respectively (Table 2).

The prevalence rate of *S. aureus* and coagulase-negative staphylococci

The overall prevalence rate of *S. aureus* versus coagulase negative staphylococci was (19.5% versus 46.5 %) in mastitic quarters and (32.1% versus 44.6%) in sub clinical mastitic quarters collected from different dairy cattle farms (Table 3).

Effect of vaccination on the prevalence clinical and subclinical mastitis in different dairy cattle farms (recovery after vaccination)

The overall prevalence rate of clinical mastitic quarters among 420 examined cows was 17.54% (286/1630) before vaccination compared to 11.8% (192/1630) after the 120 day from vaccination with a reduction of 94 cases (32.9%), however, the overall prevalence rate of sub clinical mastitis were 44.53% (726/1630) and 30.06% (490/1630) before and after vaccination, respectively with a reduction of 236 casas (32.5%) (Table 2).

The prevalence rate of *S. aureus* and coagulase-negative staphylococci isolated from subclinical and clinical mastitic quarters before and after vaccination

Before vaccination, the prevalence rete of *S. aureus* versus coagulase negative staphylococci was (19.5 % versus 46.5%) in mastitic quarters and (32.1% versus 44.6%) in subclinical mastitic quarters. On the other hand, after vaccination, the prevalence rete of *S. aureus* versus coagulase negative staphylococci was (12.5% versus 33.6%) in mastitic quarters and (18.9 versus 26.4%) in subclinical mastitic quarters (Table3).

The reduction rate of staphylococcal mastitis after vaccination with *S. aureus* **bacterin in the different dairy cattle farms**

From all examined animals regardless clinical or subclinical, *S. aureus* was reduced from 289 isolates (28.6%) before vaccination to 173 isolates (17.09%) after vaccination with *S. aureus* bacterin with a reduction rate of 40.1% (n=116 isolates). However coagulase negative staphylococci was reduced from 457 (45.2%) before vaccination to 288 isolates (28.5%) after vaccination with a reduction rate of 36.9% (n=169 isolates) (Table 4).

Effect of vaccination on total bacterial counts, total Staphylococcal counts, *Staph. aureus* counts, SCC, protein, fat, and total milk yield of bulk tank milk

After vaccination, there were reductions in the total bacterial counts, total Staphylococcal counts, *Staph. aureus* count, and BTSCC with percentages of 53.6%, 69.2%, 95.1% and 47.8% respectively. While there were increase in the total protein, fat, and total milk yield of bulk tank milk with percentages of 10.8%, 9.5% and 15.8% respectively, (Table 5 and 6).

Effect of vaccination on dairy cow culling rates per farm in different dairy farms before and after vaccination

Before vaccination, 129 (24%) cows were culled out of 537 at risk of mastitis compared to 70 out of 420 (16.6%) after vaccination with 30.8% reduction in the overall culling rate (Table 7).

The total prevalence rate of new *S. aureus* and CNS isolated from clinical and subclinical mastitic quarters of vaccinated cows in different dairy cattle farms

After vaccination, 222 new cases of clinical and subclinical mastitis were detected, from which, 108 Staphylococcal isolates were isolated. The total prevalence rates of new *S. aureus* and coagulase negative staphylococci were 10.3% (23/108) and 38.3% (85/108) (Table 8).

Rate of protection against Staphylococcal mastitis among vaccinated cows in different dairy cattle farms

The total prevalence rate of *S. aureus* before vaccination was 28.6% (289/1012) and total prevalence rate of coagulase negative staphylococci was 45.2% (457/ 222). While after vaccination, total prevalence rate of *S. aureus* was 10.3% (23/ 222) and the total prevalence rate of coagulase negative staphylococci was 38.3% (85/222) with a total protection rate of 63.9% and 15.3% for *S. aureus* and CNS, respectively (Table 8).

Table (1): Hygienic measures, milking process, management, and dry cow therapy in the different dairy farms

Item	Farm (A)	Farm (B)	Farm (C)
Bedding	Mud, straw and scraped every 6 months.	Mud, straw and scraped monthly	Dry soil, mixed with sawdust and changed every two weeks
Milking process (Machinery milking)	Pre-milking washing without drying.	The udder was washed with water and dried with a clean towel	Pre-milking washing with running water and dried with clean separate towels
Disinfectant	No disinfection during milking process	Post milking teat dipping only	Pre-milking and post milking teat dipping were used.
Dry cow therapy	No dry cow therapy	No dry cow therapy	dry cow therapy applied

Table (2): The prevalence of clinical and subclinical mastitis in the examined dairy cattle farms before and after vaccination with *Staph. aureus* bacterin

Examined farms	No. of examined animals	No. of examined quarters	Prevalence of clinical mastitis (Confirmed cases)						Prevalence of sub clinical mastitis Confirmed cases)					
			Before vaccination		After vaccination		Total reduction (Cured cases) after vaccination		Before vaccination		After vaccination		Total reduction (Cured cases) after vaccination	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Farm (A)	120	460	89	19.34%	71	15.43%	18	20.22%	253	55%	192	41.7%	61	24.1%
Farm (B)	140	550	98	17.81%	68	12.36%	30	30.6%	239	43.45%	154	28%	85	35.6%
Farm (C)	160	620	99	15.96%	53	8.5%	46	46.5%	234	37.74%	144	23.22%	90	38.5%
Total	420	1630	286	17.54%	192	11.8%	94	32.9%	726	44.53%	490	30.06%	236	32.5%

Table (3): Prevalence of *S. aureus* and coagulase negative staphylococci in the same clinical and sub clinical mastitic quarters before and after vaccination with *Staph. aureus* bacterin

Farms examined	No. of examined animals	No. of examined quarters	Clinical mastitis											Sub clinical mastitis											
			No. of cases	Before vaccination					After vaccination					No. of cases	Before vaccination					After vaccination					
				Isolated bacterial spp					Isolated bacterial spp						Isolated bacterial spp					Isolated bacterial spp					
				Total No. of isolates	*Staph. aureus*		*CNS*		Total No. of isolates	*Staph. aureus*		*CNS*			Total No. of isolates	*Staph. aureus*		*CNS*		Total No. of isolates	*Staph. aureus*		*CNS*	
					No.	%	No.	%		No.	%	No.	%			No.	%	No.	%		No.	%	No.	%
Farm (A)	120	460	89	66	21	23.6%	45	50.6%	50	15	16.8%	35	39.3%	253	217	90	35.6%	127	50.2%	150	59	23.3%	91	35.9%
Farm (B)	140	550	98	65	20	20.4%	45	45.9%	44	12	12.24%	32	32.7%	239	179	75	31.4%	104	43.5%	105	44	18.4%	61	25.5%
Farm (C)	160	620	99	58	15	15.2%	43	43.4%	38	9	9.1%	29	29.3%	234	161	68	29.1%	93	39.7%	74	34	14.5%	40	17.1%
Total	420	1630	286	189	56	19.5%	133	46.5%	132	36	12.5%	96	33.6%	726	557	233	32.1%	324	44.6%	329	137	18.9%	192	26.4%

Table (4): Reduction rate of staphylococcal mastitis after vaccination in the different dairy cattle farms

Farm	Total no. of clinical and sub clinical samples	Before vaccination		After vaccination		Reduction	
		Staph. aureus	*CNS*	*Staph. aureus*	*CNS*	*Staph. aureus*	*CNS*
Farm(A)	342	111 (32.5%)	172 (50.29%)	74 (21.6%)	126 (36.8%)	37 (33.3%)	46 (26.7%)
Farm(B)	337	95 (28.2%)	149 (44.2%)	56 (16.6%)	93 (27.6%)	39 (41.05%)	56 (37.6%)
Farm(C)	333	83 (24.9%)	136 (40.8%)	43 (12.9%)	69 (20.7%)	40 (48.2%)	67 (49.3%)
Total	1012	289 (28.6%)	457 (45.2%)	173 (17.09%)	288 (28.5%)	116 (40.1%)	169 (36.9%)

Table (5): Comparison between total bacterial, staphylococcal and *S. aureus* counts in bulk tank milk before and after vaccination of dairy cattle with *Staph. aureus* bacterin

Farm	TBC of BTM CFU/ml			Total staphylococcal count of BTM CFU/ml			Total *Staph. aureus* counts of BTM CFU/ml		
	Before vaccination	After vaccination	Reduction	Before vaccination	After vaccination	Reduction	Before vaccination	After vaccination	Reduction
Farm (A)	2×10^{6}	94×10^{4}	53%	4000	1400	65%	300	25	91.6%
Farm (B)	4.5×10^{5}	21.6×10^{4}	52%	1500	430	71.3%	220	10	95.5%
Farm (C)	3.1×10^{5}	12.4×10^{4}	60%	800	110	86.3%	200	Zero	100%
Total	2.76×10^{6}	128×10^{4}	53.6%	6300	1940	69.2%	720	35	95.1%

Table (6): Comparison between somatic cell counts, fat and protein percentages, and average of milk yield average per cow in bulk tank milk before and after vaccination with *Staph. aureus* bacterin

Examined farm	SCC of BTM			Average of bulk tank fat and protein percentage						Average of milk yield		
				Fat %		Protein %		Total increase				
	Before	After	Reduction	Before	After	Before	After	Fat%	Protein%	Before	After	Total increase
Farm(A)	716,371/ ml	394,004/ ml	45%	3.7	4	3.2	3.5	7.3%	8.5%	22.8K.g	26K.g	14.03%
Farm(B)	441,761/ ml	220,880/ ml	50%	3.8	4.2	3.3	3.7	9.5%	%10.8	23.2K.g	27K.g	16.3%
Farm(C)	398,262/ ml	198,285/ ml	50.2%	3.9	4.4	3.5	4	11.4%	12.5%	24K.g	28.1K.g	17.08%
Average	518,798/ ml	271,056/ ml	47.8%	3.8	4.2	3.3	3.7	9.5%	10.8%	70K.g	81.1K.g	15.8%

Table (7): Effect of vaccination on dairy cow culling rates per farm before and after vaccination with *Staph. aureus* bacterin

Farm	Cows before vaccination			Vaccinated cows			Total reduction
	No. of cows at risk	No. of culled cows	percentage	No. of cows at risk	No. of culled cows	percentage	percentage
Farm (A)	153	43	28.1%	120	23	19.16%	31.8%
Farm (B)	183	46	25.1%	140	25	17.5%	%30.3
Farm (C)	201	38	18.9%	160	22	13.75%	27.6%
Total	537	129	24%	420	70	16.6%	30.8%

Table (8): The prevalence rate of *S. aureus* and CNS isolated from clinical and subclinical mastitic quarters from unvaccinated dairy cattle and new cases after vaccination with *Staph. aureus* bacterin

Staphylococcal mastitis cases before vaccination							New Staphylococcal mastitis cases after vaccination						Rate of protection	
Farm			*S. aureus*		CNS				*S. aureus*		CNS			
	Total no. of clinical and sub clinical samples	Total no. of isolates from clinical and sub clinical quarters	No.	%	No.	%	Total no. of clinical and sub clinical samples	Total no. of isolates from clinical and sub clinical quarters	No.	%	No.	%	% of *S. aureus*	% of CNS
Farm (A)	342	283	111	32.5%	172	50.3%	52	28	7	13.5%	21	40.3%	58.5%	19.9%
Farm (B)	337	244	95	28.2%	149	44.2%	77	39	9	11.8%	30	38.9%	58.2%	11.9%
Farm (C)	333	219	83	24.9%	136	40.8%	93	41	7	7.5%	34	36.6%	69.8%	10.3%
Total	1012	746	289	28.6%	457	50.3%	222	108	23	10.3%	85	38.3%	63.9%	15.3%

Discussion:

Mastitis is considered one of the costliest diseases affecting dairy cattle worldwide during lactation seasons as it lowers milk yield, affects milk quality, and is the largest reason for antibiotic consumption in dairy farms [11]. Subclinical mastitis is very common in dairy cows because of insufficient knowledge between farmers as most of them did not even know that subclinical mastitis is found [12]. The high prevalence rates of mastitis were also related to the lack of efficient milking hygiene procedures, as most of the bacterial findings were contagious pathogens.

The present study gives information about prevalence rates of *Staphylococcal* mastitis in lactating cows in different dairy herds in Egypt and how to control and reduce the economic losses due to *S. aureus* mastitis.

The variation in the prevalence rate of mastitis among studied farms might be due to different risk factors like management, environmental and hygienic measures, animal risk factors, causative agents, and lack of awareness of farmers to the losses caused by mastitis [1].

The overall prevalence rate of clinical mastitis at the level of the quarter was 17.54%. These findings are in close alignment with the results of Tilahun and Aylate [13] in Ethiopia who reported clinical mastitis in 288 (16.2%) quarters out of 1776 quarters examined clinical mastitic quarters were 288 (16.2%) and lower than results obtained by El-Damaty, [14] in Egypt who found that the overall prevalence rate of clinical cases at the level of the quarter was 20.5%.On the other hand, it was noticed that the overall prevalence rate of subclinical mastitis in the examined quarters of dairy cattle was 44.53% which is close with the findings of Karimuribo *et al*., [15] who reported that prevalence of subclinical mastitis in lactating cows in small farms of Tanzania was 46.2% at the quarter level. However, it is lower than findings of Belina *et al.*, [16], Kifle and Tadele, [17], Birehanu, [18] and El-Damaty, [14] who reported that the prevalence rate of subclinical mastitis at the level of the quarter were 50.2%, 63.1%, 52.4%, and 51.6%, respectively. The high prevalence of subclinical mastitis may be due to improper milking hygiene practices, lack of post milking teat dipping, milking of animals with clinical mastitis before the healthy ones and the difficulty of detecting sub-clinical mastitis by the owners [1].

Regarding the incremented bacteria in clinical mastitic quarters, overall prevalence rate of *S. aureus* was 19.5% which is following El-Damaty, [14] who isolated *S. aureus* from 21.3% in clinical mastitic quarters. On the other hand, the overall prevalence rate of coagulase-negative staphylococci was 46.5% which differs from the finding of Bitew *et al.,* [19] detected coagulase-negative staphylococci in 51.9% examined mastitic quarters. In current study *S. aureus* was isolated from 32.1% of the subclinical mastitic quarters which is similar to 31.9% reported by El-Damaty, [14]. On the other hand, the overall prevalence rate of CNS was 44.6% which is lower than 54.7% and 56.2% reported by Abrahmsen *et al.*, [20] in Uganda and Bitew *et al.*, [21] in Ethiopia, respectively.

Mastitis is a complex disease affected by several factors as management, hygienic measures, environmental conditions, and causative agents so its prevalence rate will vary. This variation in the prevalence rate of mastitis among studied farms might be due to different risk factors like management, environmental and hygienic measures, animal risk factors, causative agents, and lack of awareness of farmers to the losses caused by mastitis [1]. In farm (A) the present study found that the prevalence rate of *S. aureus* versus coagulase-negative staphylococci in clinical and sub-clinical quarters was 32.5% versus 50.29%, which is the highest prevalence between the three farms. In farm (B), the overall prevalence rate of *S. aureus* isolated from milk samples was 28.2% compared to 44.2% for coagulase-negative staphylococci. In farm (C) the overall prevalence rate of *S. aureus* isolated was 24.9% compared to 40.8% for coagulase-negative staphylococci. The reason for this high prevalence is poor udder hygiene, absence of dry period treatments, bad farming management (bedding material was mud, straw, and not scraped), imperfect cleaning of the milking area after the milking, no udder cleansing tissues before milking, lack of teat dipping after milking and no dry cow therapy. However, low prevalence in farm (C) because bedding material was dry soil, sometimes mixed with sawdust, changed every two weeks at least ,using pre-milking and post milking teat dipping is applied and dry cow therapy with Ceftiofur hydrochloride (Spectramast® DC which is used for the treatment of subclinical mastitis at the time of dry off in dairy cattle associated with *Staph. aureus*, *Strept. dysgalactiae* and *Strept. uberis.*, Zoetis Inc. Kalamazoo, MI 49007) at dry period .

Our results are following the findings of Banerjee *et al.*, [22] and Grewal *et al.*, [23] who reported a high prevalence rate of *S. aureus* which maybe since the principal reservoirs of *S. aureus* is the udder skins, milk of the infected udder and can transfer from the udder of infected cows to healthy cows via milker's hands, utensils, towels, and the environment (Floor) in which the cows were kept with the ability to penetrate the mammary tissue resulting in deep-seated foci protected by barriers [24, 25]. Smith, [26] reported that *S. aureus* is the most etiological agent of both clinical and sub-clinical mastitis and can resist antibiotic treatment and recur chronically. *S. aureus* can survive under wide extremes of temperature and moisture and also colonizes teat orifices, damaging roughened epithelium which makes it to be the most frequently isolated pathogen [27, 28]. *S. aureus* can reside intracellularly and establish a chronic infection that can persist for the life of the animal due to abscesses formation around these bacteria. It is also considered a major problem for dairy cattle as it produces a broad spectrum of surface components (proteins and capsular polysaccharides) and exotoxins which play an important role in the pathogenesis of bovine mastitis as these toxins are injurious to milk-producing cells, impair mammary gland and immune defense mechanisms [29].The highest prevalence (32.5%) of *S. aureus* from mastitic and subclinical mastitic quarters in the current study alarms that these pathogens are an etiological agent of major concern in clinical and subclinical mastitis of lactating cows in the different dairy farms.

Several types of research on mastitis vaccines have been carried out for 30 years and several mastitis vaccines have been produced. The main aim of this study is to determine the effect of *S. aureus* bacterin in controlling staphylococcal mastitis in dairy cattle. Evaluation parameters were conducted to evaluate the response of vaccination with *Staph. aureus* bacterin in cows based on clinical signs, milk production, somatic cell count, shedding of *S. aureus* in milk and antigen-specific IgG in blood [30].

Staph. aureus bacterin was given to lactating cows and was found to improve clearance rates of existing *S. aureus* mastitis, but it has little effect on reducing new mastitic cases [31]. However, *S. aureus* bacterin was found to be effective in preventing new infections with *S. aureus* and coagulase-negative staphylococci in dairy heifers, and minimizing somatic cell count, and increasing milk yield [32, 33].

In the current study, after vaccination the reduction rates in the prevalence of clinical and sub clinical mastitis than before vaccination were 32.9% and 32.5% respectively. Moreover, *S. aureus* and coagulase-negative staphylococci were respectively reduced in all examined animals regardless clinical or subclinical with percentages of 40.1% and 36.9% that before vaccination. This result is in close with the findings of Schukken *et al.*, [34] who reported that bacterin of *S. aureus* reduced CNS mastitis and *S. aureus* mastitis with percentages of 35% and 45%,respectively . Besides that, Nickerson *et al.,* [32] found that vaccination reduced coagulase-negative staphylococcal mastitis (which became chronic) by about 30%. Also, Pankey *et al.,* [31] founded that 73% of the *S. aureus* IMI cured spontaneously as compared to 40% in the control group when he studied the efficacy of commercial *S. aureus* vaccine (Lysigin) and concluded that it will increase the spontaneous cure rate against *Staph. aureus* IMI and lower SCC but not prevent new IMI in adult cows, he also suggested that the increased incidence of spontaneous cure and lower incidence of clinical mastitis within vaccinated cows during lactation was due to enhanced natural defense mechanisms. However, Nickerson *et al.,* [33] mentioned that the Lysigin® vaccine was shown to be effective in preventing new *S. aureus* IMI when administered to bred dairy heifers. Leitner *et al.,* [35] found that the use of polyvalent bacterins in cows with chronic mastitis caused by *S. aureus* also yields encouraging results, in a field trial, a group of cows with chronic *S. aureus* infections were vaccinated with a polyvalent bacterin, whereas another group received only a placebo at the end of the 348-day trial, 30% of the vaccinated cows were considered cured, compared with only 6% of the control cows.

Results of vaccination against *S. aureus* to control *S. aureus* mastitis were with variable success [32, 36- 39]. Results of reduced rates of *S. aureus* mastitis ranged from 45 to 65 % for experimental vaccines [37, 38, 40], the variability of results from field trials may be due to variations in farm management practices [41].

The total bacterial count, total staphylococcal count, and *Staph. aureus* counts were reduced by 53.6%, 69.2%, and 95.1%, respectively in the current study that agree with Calzolari *et al.,* [42] and Watson *et al.,* [43] who reported that *Staph. aureus* counts in infected quarters and somatic cell counts of milk of vaccinated cows and that the vaccine was effective in decreasing new *S. aureus* IMI.

Somatic cell counts are used to evaluate the general health status of the udder of lactating cows, in our study the mean SCC (over the120 days of vaccination) was lower after vaccination in lactating cows by 47.8% which in close agreement with Nickerson *et al.,* [32] who found that the somatic cell count was minimized by 50% in vaccinated cows compared with controls. A reduction in somatic cell counts was observed in a study done by Leitner *et al.,* [8] who found that the SCCs for the 348 d after vaccination with *S. aureus* bacterin against *S. aureus* mastitis vaccinated cows and heifers had a slightly lower somatic cell count than the controls $310 \pm 19 \times 10^3$ cells/ml compared with $324 \pm 21 \times 10^3$ cells/ml.

Our results showed that both fat and protein percentage of bulk tank milk in different dairy farms after vaccination were increased by 9.5% and 10.8%, respectively. Likewise, Nickerson et al., [33] found that the 305-day pounds of both fat and protein production were higher in vaccinated cows compared with controls by 20.3% and 4.8%, respectively. Also, the total average of milk yield per cow in the different dairy farms after vaccination increased by 15.8%, the increase in milk production per day in vaccinated cows during lactation may be resulted from the overall improvement of udder health status in vaccinated cows. These results agree with Leitner *et al.,* [8] who founded that there were significant differences in milk production among vaccinated cows; in all herds, the vaccinated cows had higher milk production than the controls. Also, Athar, [44] and Pellegrino *et al.,* [30] reported an increase in daily milk production after vaccination with using inactivated polyvalent vaccines against *S. aureus* mastitis. A study made by Nickerson *et al.,* [33] reported an approximate 10% increase in milk production in vaccinated compared with controls.

The prevalence rate of new *Staph. aureus* cases in clinical and subclinical mastitis at different dairy farms after vaccination was 10.3% with a protection rate of 63.9% , which is in close agreement with Nickerson *et al.*, [32, 33] who used Lysigin previously in lactating cows and was found to be 45–60% effective in preventing *Staph. aureus* mastitis at time of calving.

Vaccine efficacy was observed to be fluctuated depending on farm-specific characteristics, such as strain types [45, 46] and farm management practices [47], as we identified significant differences between farms. Increasing efficacy of the vaccine should be in barrel with good farm management practices included treatment protocols, segregation and culling of known infected animals, milking procedures, disinfection of milking equipment and kind of the used disinfectant [48, 49]. For example, on-farm with good management practices (Farm C), *S. aureus* reduced from 24.9% to 7.5% with protection rate of 69.8%. In this farm, mastitis control practices including proper washing of the udder before milking, good hygienic practices, rapid culling of cows with recurrent mastitis, pre and post-teat dipping were all applied. Studies explained that pre and post teat dipping decreases the spread and transmission of mastitis from infected cows to healthy ones, also the application of dry cow therapy decreases the reservoir, which in turn decreases the further exposure of the teat to pathogenic bacteria [50- 52]. Vaccination against *S. aureus* showed a reduction in the prevalence rate of *S. aureus* infection; however, *S. aureus* remains endemic despite vaccination. In the present study, we did not perform a cost-benefit analysis of the vaccine regimen used in this field study. Eventually, such a cost-benefit analysis will be essential to decide under what infection conditions vaccination would be economically beneficial to the farm.

Conclusion

Vaccination of cows (five ml of *S. aureus* bacterin) by two doses with 14-day interval decrease the severity of clinical mastitis, reduce the prevalence of *S. aureus* and coagulase-negative staphylococcal mastitis. Also, total bacterial count, somatic cell count, total Staphylococcal count, and *S. aureus* count was reduced. Also, vaccine efficacy was stretched to fat, protein production and milk yield were elevated.

Conflict of Interest

The authors declare no conflict of interest

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الملخص العربي

التقييم الحقلي لاستخدام لقاح الاستافيلوكوكس أوريس في مزارع الحلاب

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أجريت هذه الدراسة على 420 بقرة حلاب من ثلاث مزارع مختلفة فى محافظتي دمياط والشرقية في الفترة من مايو 2015 وحتى ديسمبر2017 .تم حقن لقاح اللايسيجين بجرعة 5 سم عضل لكل بقرة وتكراره بنفس الجرعة بعد 14 يوم من الجرعة الأولى (كجرعة تنشيطية)، وعلى مدار 120 يوم تمت متابعة تأثير اللقاح على الحالات الإكلينيكية والكامنة وتأثيره على نسبة الإصابة بالمكورات العنقودية الذهبية والمكورات العنقودية السالبة لاختبار التجلط ، وكذلك تأثيره على الخلايا الجسدية والعدد البكتيري وعدد المكورات العنقودية الذهبية و نسبة البروتين والدهون في اللبن وكذلك إنتاجية كل بقرة،أظهرت النتائج أن العدد الكلي للأرباع المصابة بالتهاب الضرع الإكلينيكي قبل التحصين كانت 286 بنسبة 17.54% بينما بعد التحصين العدد الكلى للأرباع المصابة بالتهاب الضرع الإكلينيكي انخفض إلى 192 بنسبة 11.8%. بينما كان العدد الكلى للأرباع المصابة بالتهاب الضرع الكامن قبل التحصين 726 بنسبة 44.53%، بينما بعد التحصين العدد الكلى للأرباع المصابة بالتهاب الضرع الإكلينيكي انخفضت إلى 490 بنسبة 30.06 %.كما أوضحت النتائج قبل التحصين بين الأرباع المصابة بالتهاب الضرع الإكلينيكي كانت نسبة الإصابة بميكروب المكورات العنقودية الذهبية 19.5% أما نسبة الإصابة بميكروب المكورات العنقودية السالبة لاختبار التجلط كانت 46.5% ، بينما بعد التحصين انخفضت نسبة الإصابة بميكروب المكورات العنقودية الذهبية إلى 12.5% أما نسبة الإصابة بميكروب المكورات العنقودية السالبة لاختبار التجلط انخفضت إلي 33.6%. وأيضا أوضحت النتائج قبل التحصين بين الأرباع المصابة بالتهاب الضرع الكامن كانت نسبة الإصابة بميكروب المكورات العنقودية الذهبية 32.1% أما نسبة الإصابة بميكروب المكورات العنقودية السالبة لاختبار التجلط كانت 44.6%، بينما بعد التحصين انخفضت نسبة الإصابة بميكروب المكورات العنقودية الذهبية إلى 18.9% أما نسبة الإصابة بميكروب المكورات العنقودية السالبة لاختبار التجلط انخفضت إلى 26.4%.كما أظهرت الدراسة أن الخلايا الجسدية قبل وبعد التحصين انخفضت بنسبة 47.8% وانخفض العدد البكتيري بنسبة 53.6%، كما انخفض عدد البكتيريا العنقودية بنسبة 69.2%، وأيضا انخفض عدد المكورات العنقودية الذهبية بنسبة 95.1%، وكذلك انخفضت إقصاء الأبقار والتي سببها التهاب الضرع بنسبة 30.8%. وظهر أيضا تأثير ملحوظ للقاح اللايسيجين على معدل انتاج اللبن والذى ارتفع بنسبة 15.8% وأيضا الدهون والبروتين حيث ارتفع كل منهما بنسبة 9.5% و 10.8% على التوالى