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ARTICLE

Meta-Analysis on Efficacy of Vaccination against *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

Mastitis is a common disease responsible for the biggest economic loss in the dairy industry. Antibiotic therapy does not provide long-term protection. And residue is a major concern in food safety. Vaccination is an alternative control method with great potential for bovine mastitis. Our study focus on evaluating vaccine efficacy regarding reducing the incidence of clinical and subclinical mastitis. Meta-analysis was used to pool data extracted from previous studies. 26 records from 13 studies were examined. A fixed effect model was constructed assigning incidence as the measurement of the outcome. Risk ratio (RR) was the parameter that measured the incidence differences between treated group and control group. Studies and records were categorised based on vaccine antigens. In vaccine against *Staphylococcus aureus*, RR was 0.76; 95% CI (0.65,0.89), while in vaccine against *Escherichia coli* RR was 0.96; 95% CI (0.86,1.08).

1. Introduction

Mastitis is inflammation in quarters of the udders and the most significant cause of economic loss in dairy industry which manifests as reduced milk yield, milk composition change, reduced quality, and compromised reproduction capacity [1]. Current mainstream control method is prepartum antibiotic therapy with very limited long-term effects in reducing somatic cell counts (SCC) and increased milk yield. Bacterial antibiotic resistance and drug residues in dairy products could be the inherent risks. Vaccination function as a control method by generating long lasting immunity against offending pathogens in dairy cow, and does not have withdrawal time, which makes it a promising alternative protocol to the established ones [2,3,4]. Vaccination could be helpful as an aid for prevention of a few bacteria.

The search for effective vaccine can trace back to last century and positive result was not reported until 1980s. A vaccine, combining heat-killed capsular-type A and B *Staphylococcus aureus* and capsular polysaccharide, was reported to increase the resistance against *Staphylococcus aureus* in dairy cows, and mitigated mastitis related yield reduction [5]. About the same time, the investigation on vaccine against *Escherichia coli* also produced some positive results. J5 antigen was proven to reduce incidents of clinical coliform mastitis [6]. However, conflicting results were also reported. Some researchers argued that

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vaccines had no effect on alleviating clinical parameters like incidence rate, SCC and commercial parameters like yield loss and culling rate [7,8,9,10]. The failure of vaccine in combating mastitis could be attributed to the multiple causative pathogens [8,11], low mammary antibody titer [12], environmental factors and ect.

A previous report proposed that the implement of vaccination had some advantages [12]. With the release of the new commercial vaccine and data from new clinical trials, it is necessary to re-evaluate the mastitis vaccine efficacy and its potential application.

2. Methods

2.1 Literature Search Strategy

The literature search was conducted in Pubmed electronic database, Web of Science, in January 2018. The Medical Subject Heading (Mesh) and keywords for disease and intervention were searched in [All Fields] using various combinations. The search terms for disease were mastitis OR "bovine mastitis" and search terms for intervention were vaccine* OR vaccination*. For example, the searching strategy in Pubmed was (((Mastitis"[Mesh]) OR "Mastitis, Bovine"[Mesh])) AND (("Vaccination"[Mesh]) OR "Vaccines"[Mesh]). To supplement computer search, a manual search for possible articles was also conducted by searching references of identified articles.

The searching result was imported into Endnote X8. Duplicates were eliminated by cross check author's name, article's title and the publication year.

2.2 Inclusion and Exclusion Criteria

Studies using controlled trials and observational method like cohort study and case-control study were included. Controlled trails must consist both randomized controlled trials (RCT) and controlled clinical trials (CCT). Other inclusion criteria are: (1) participant of the study were dry or lactating cows; (2) intervention was vaccination; (3) control groups were unvaccinated or treated with placebo; (4) outcomes must include the incidence of mastitis; antibiotic treatment as control group.

2.3 Date Extraction

Trial duration, vaccination regimens and outcome measure methods were extracted. In studies containing multiple trials, if summation was available, the data in summation were extracted and all trials were considered as one study; if summation was not available, multiple-center trial was considered as one study or the data of trials were extracted separately.

2.4 Quality Assessment

The quality of RCT was assessed for the risk of bias by the Cochrane Collaboration's tool. Cochrane Collaboration's tool contains following items: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias. The first and second items assess selection bias. The third and fourth items assess performance bias. The fifth item assesses detection bias and the sixth item assesses reporting bias.

The quality of CCT was assessed by MINORS (methodological index for non-randomized studies) [13]. MINORS is particularly suitable for the evaluating non-randomized studies. There are 12 items measured in 0~2 (0 for no related report, 1 for insufficient report, 2 for sufficient report). The top eight items were for the uncontrolled groups and the rest were for the study with control groups. The last item was excluded because it assesses the statistical analysis method, while in our study, the extracted data were re-analyzed to calculate RR.

2.5 Assessment of Risk of Bias

Depending on the type of a trial, various tools were used to assess risk of bias.

2.6 Statistic Analysis

Risk ratio was used to estimate the outcome of each trial. We used STATA 14.0 to pool results with a fixed effect model. Heterogeneity was evaluated by Chi2 and I2. If I2 is below 25%, then it indicates low heterogeneity. If I2 is above 75%, it indicates high heterogeneity. When high heterogeneity was observed, data were pooled by adopting a random model. Sensitive analysis was conducted by omitting one study each time, evaluating the influence of each study on the overall effect size (RR) and the source of heterogeneity. Bias was assessed and presented by a funnel plot. The symmetry of the funnel plot revealed the extent of bias. A study with no bias is perfectly symmetrical. We included both subjective visual assessment and the objective Egger's test. The latter was conducted via the meta command in STATA 14.0 to quantitatively assess symmetry of the funnel plot and publication bias. If the p-value in each test was below 0.1, the plot was deemed asymmetric.

A subgroup analysis was conducted to evaluate the influence of the sample size. Datum was divided into two subgroups based on the calculated result of the sample size at the beginning of the analysis. The formula was shown below:

$$n = 2\frac{\left(\mu_\alpha + \mu_\beta\right)^2 P(1-P)}{(P_1 - P_0)^2}$$

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$$P = \frac{P_0 + P_1}{2}$$

P_0 was the incidence in vaccinated group. P_1 was the incidence in control group. μ_a and μ_b were t-value of type one error and type two error when df=∞.

3. Results

The literature search in electronic databases initially identified 323 studies, 279 studies were from Pubmed, 34 studies were from Sciencedirect and 10 studies from manual search. 10 duplications were eliminated. 245 studies were excluded after we examined their titles and abstracts. Then full-article sift-selection was conducted on the remaining 68 studies to verify eligibility. 58 studies were excluded for following reasons: 25 studies measured the outcome in methods incompatible to our established criteria such as the concentration of antibodies, SCC, milk yield, pathogens in milk samples and etc. 4 studies were conducted in species other than bovine, like goats and mice. 2 studies did not utilize vaccination as intervention. 2 studies had no control groups. 1 study was case control study. 8 studies were experimental challenge trial. Data in 12 studies could not be extracted. 2 studies have other incompatible objectives (Figure 1).

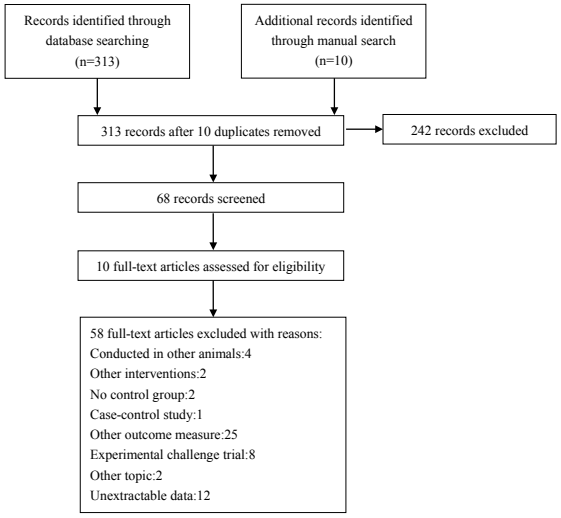

Figure 1. Flow chart search strategy

The selected studies presented the outcome with two methods, three were measured by prevalence while seven were measured by the number of cases. The duration of date recording ranged from 30DIM to 120DIM. The characteristics of the records were shown in Table 1. Different vaccination protocols were used, six of them used label regime while others used modified protocols (Table 2). The data extracted was represented in table form (Table 3a, 3b).

Table 1. Characteristics of studies included in meta-analysis

First Authors	Origin of Vaccine	Type of Control Group	Outcomes measures	Study Type
Guccione (2017,a)	Startvac	Blank Control	1.Incidence of Clinical Mastitis 2. Incidence of Subclinical Mastitis	CCT
Guccione (2017,b)	Startvac	Blank Control	1. Incidence of Clinical Mastitis 2. Incidence of Subclinical Mastitis	CCT
Bradley (2015,a)	Startvac	Blank Control	Incidence of Clinical Mastitis	RCT
Bradley (2015,b)	Startvac	Blank Control	Incidence of Clinical Mastitis	RCT
Morimoto (2011)	*E. coli*	Blank Control	Incidence of Clinical Mastitis	CCT
Wilson (2007)	*E. coli*	Blank Control	Incidence of Clinical Mastitis	CCT
Tenhagen (2001)	*S. aureus*	Placebo	Incidence of Clinical Mastitis	CCT
Hodemaker (2000)	*S. aureus*	Placebo	1.Incidence of Clinical Mastitis 2.Incidence of Subclinical Mastitis	RCT
Waston (1996,a)	*S. aureus*	Blank Control	1. Incidence of Clinical Mastitis 2.Incidence of Subclinical Mastitis	RCT
Waston (1996,b)	*S. aureus*	Blank Control	1. Incidence of Clinical Mastitis 2.Incidence of Subclinical Mastitis	RCT
Nordhaug (1994)	*S. aureus*	Placebo	Incidence of Clinical Mastitis	RCT
Mc Clure (1994)	*E. coli*	Blank Control	Incidence of Clinical Mastitis	RCT
Gonzailez (1989)	*E. coli*	Blank Control	Incidence of Clinical Mastitis	RCT

Table 2. Immunization protocols of studies included in meta-analysis

First Authors	Immunization Procotol	Immunization Route
Guccione (2017,a)	45 and 10 days before the estimated date of calving	Intramuscular Injection
Guccione (2017,b)	Label regime	Intramuscular Injection
Bradley (2015,a)	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.	Intramuscular Injection
Bradley (2015,b)	Label regime	Intramuscular Injection
Morimoto (2011)	On the day of recruitment (d 0), 30 later (d 30)	Subcutaneous Injection
Wilson (2007)	Before cows were dried off to end the previous lactation and again at 21 to 28 days before the calving due date	Subcutaneous Injection
Tenhagen (2001)	5 and 2 weeks before the estimated date of calving	Subcutaneous Injection
Hodemaker (2000)	5 and 2 weeks before the estimated date of calving	Subcutaneous Injection

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Watson (1996,a)	The last trimester of pregnancy	Intramuscular Injection
Watson (1996,b)	The end of the previous lactation	Intramuscular Injection
Nordhaug (1994)	8 and 2 weeks before the estimated date of calving	Subcutaneous Injection
Mc Clure (1994)	The first injection at drying off the second one at 2 or 3 weeks before calving	Intramuscular Injection
Gonzailez (1989)	The first injection at drying off, the second one in 28 days later the third one within 14 days after calving.	Subcutaneous Injection

Label regime: the first injection, 45 days before the predicted calving; the second injection, 35 days later (10 days before the predicted calving date); the third injection, 62 days later (52 days after the predicted calving date)

Table 3a. Date extracted from studies (vaccine against *Staphylococcus aureus*), including duration, the number of bovines in vaccinated group and control group

First Author	Duration	Vaccinated Group			Control Group		
		+	-	Total	+	-	Total
Clinical Mastitis							
Guccione(2017,a)	3M	2	28	30	2	28	30
Guccione(2017,a)	3M	0	30	30	0	30	30
Bradley(2015,a)	4M	8	550	558	8	568	576
Bradley(2015,b)	4M	10	405	415	8	568	576
Tenhagen(2000)	3M	67	97	164	74	83	157
Hodemaker(2000)	—	5	30	35	6	30	36
Watson(1996,a)	9M	41	634	675	62	663	725
Watson(1996,b)	9M	4	167	171	5	149	154
Nordhaug(1994)	—	9	49	58	10	40	50
Subclinical Mastitis							
Guccione(2017,A)	3M	4	26	30	3	27	30
Guccione(2017,B)	3M	6	24	30	12	18	30
Hodemaker(2000)	—	19	16	35	16	20	36
Total		202	2219	2421	268	2352	2620

Table 3b. Date extracted from studies (vaccine against *Escherichia coli*), including duration, the number of bovines in vaccinated group and control group

First Author	Observation Time	Vaccinated Group			Control Group		
		+	-	Total	+	-	Total
Clinical Mastitis							
Bradley (2015,a)	4M	63	495	558	57	529	576
Bradley (2015,b)	4M	48	367	415	57	529	576
Morimoto (2011)	10M	54	181	235	50	195	245
Subclinical Mastitis							
Wilson (2007)	20M	27	224	251	15	291	306
Mc Clure (1994)	5M	49	597	641	78	568	646
Gonzailez (1989)	—	6	227	233	29	198	227
Total		429	2501	2930	470	2706	3156

Note: M = month

Six RCT were included in the analysis. The result of quality evaluation was presented in Figure 2. Three out of six studies conducted random sequence generation and were evaluated as low risk [14,15,16]. Among these three studies, two utilized random number table and one utilized coin flipping. The rest did not report relevant information and therefore were deemed as unclear risk [17,7,9].

Figure 2. Qualification assessment of studies included

None of these six studies reported relevant information of whether allocation concealment was applied, therefore all of them were deemed as unclear risk.

Four studies completed blinding of participant and personnel [16,14,8,9]. Among these four studies, one coded vaccine and placebo in order to bind participant and personnel and other three studies reported clearly that participant and personnel knew nothing about allocation. The rest two studies provided no information relevant to this evaluation item and therefore were deemed as unclear risk [15,17].

Six studies completed blinding of outcome assessment. The participant and personnel of the four studies men-

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tioned above also conducted outcome assessment and therefore were evaluated as low risk. Other two studies assessed the outcomes by clinical symptoms, SCC, bacterial culture and therefore the process of the outcome assessment were considered objective.

All six studies reported complete outcome data. Information regarding exclusion of participants during the experiment and its reason was presented clearly. All six studies have no bias of selective reporting or bias of other types.

Four CCT were included. The result of quality assessment was shown in the table 4. The total scores ranged from 13 to 17. For 6th item, four out of six studies scored 1 for the lack of explicit standards of time monitoring. Only one study conducted objective evaluation of mastitis. No study estimated sample size.

Table 4. Qualification assessment of studies included using MINORS

Author	○1	○2	○3	○4	○5	○6	○7	○8	○9	○10	○11	total
Guccione (2017)	2	2	2	2	2	1	2	0	0	2	2	17
Morimoto (2011)	2	2	2	1	0	1	2	0	0	2	2	14
Wilson (2007)	2	2	2	0	0	1	2	0	0	2	2	13
Tenhagen (2001)	2	2	2	0	0	1	2	0	2	2	2	13

The pathogen in seven studies was *Staphylococcus aureus*. Twelve groups of datum were extracted from these seven studies. 4530 cows were included after pooling, 2166 of them were vaccinated, and 2364 received placebo or no treatment. One group of datum was excluded because the incidence in both vaccinated and control group was zero, and RR was not available. Low heterogeneity between was observed with I2=0% (Figure 3). The overall RR was 0.86(0.72, 1.02).

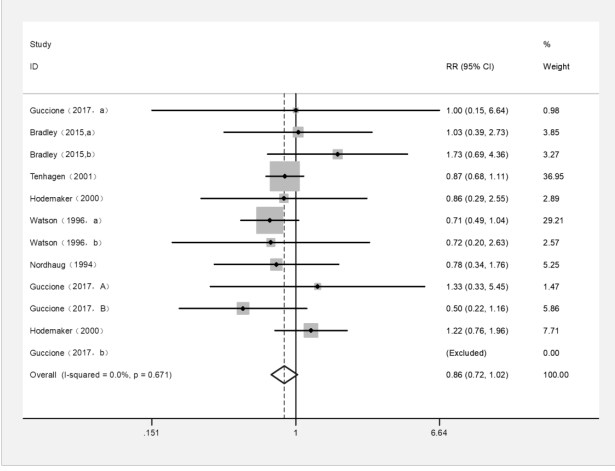

Figure 3. A forest plot of risk ratios and 95% confidence intervals for 12 records assessing the efficacy of *Staphylococcus aureus* vaccines

The pathogen in five studies was *Escherichia coli*. Six groups of datum were extracted from these seven studies. 4914 cows were included after pooling, 2338 of them were vaccinated, and 2576 cows received placebo or no treatment. High heterogeneity between was observed with I2=82.8% (Figure 4). The overall RR was 0.96 (0.81, 1.12).

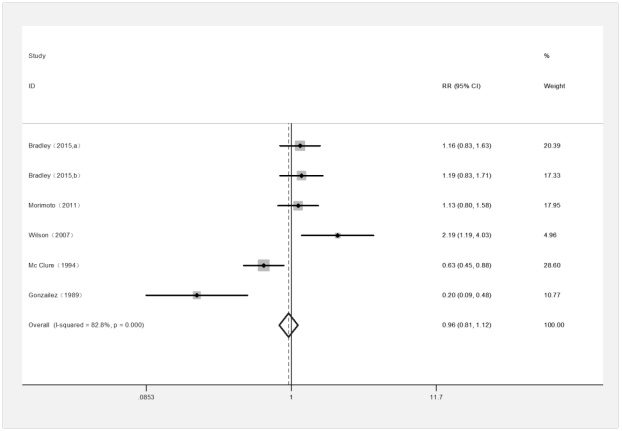

Figure 4. A forest plot of risk ratios and 95% confidence intervals for 6 records as-sessing the efficacy of *Escherichia coli* vaccines

Each dot in the funnel plot represents a set of data. Data with larger sample size were allocated to the higher position on the diagram. The funnel plot of *Staphylococcus aureus* vaccine was visually symmetrical (Figure 5), while the funnel plot of *Escherichia coli* vaccine was visually asymmetrical (Figure 6). The result of Egger's test was consistent with visual assessment, with p-value 0.642 and 0.614 respectively (Table 5,6).

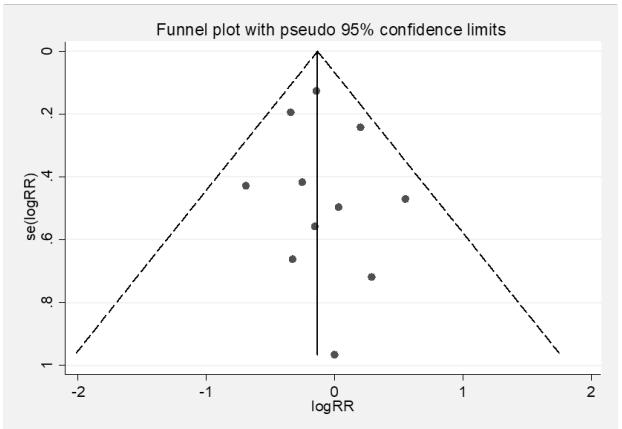

Figure 5. A funnel plot illustrating the deviation of meta-analysis assessing the efficacy of *Staphylococcus aureus* vaccines

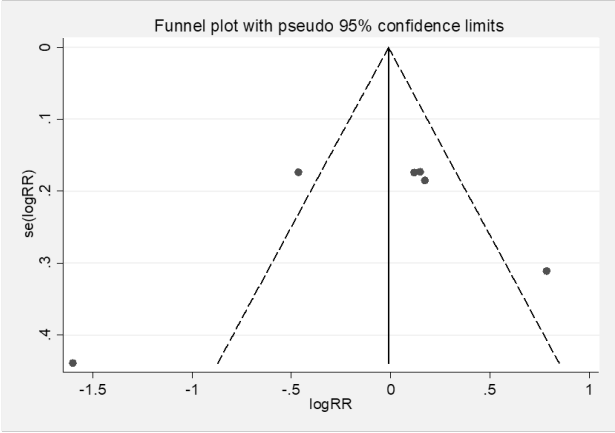

Figure 6. A funnel plot illustrating the deviation of meta-analysis assessing the efficacy of *Escherichia coli* vaccines

Based on the parameters set in the previous study, the incidence of mastitis in vaccinated group and control group was assumed as 0.125 and 0.25 respectively, the probability of testing type one error was assumed as 10%(α=0.1), the probability of testing type two error was assumed as 80%(1-β), therefore the μ_a and μ_b was 1.645 and 1.282. So the minimum sample size was 167.

The subgroup analysis revealed that sample size had no influence on the conclusion of the efficacy of the *Staphylococcus aureus* vaccine with RR 0.87(0.72, 1.07) and 0.86(0.72, 1.02) respectively (Figure 7). The sample size of all study in *Escherichia coli* vaccine group was larger than 167, thus subgroup analysis was not conducted.

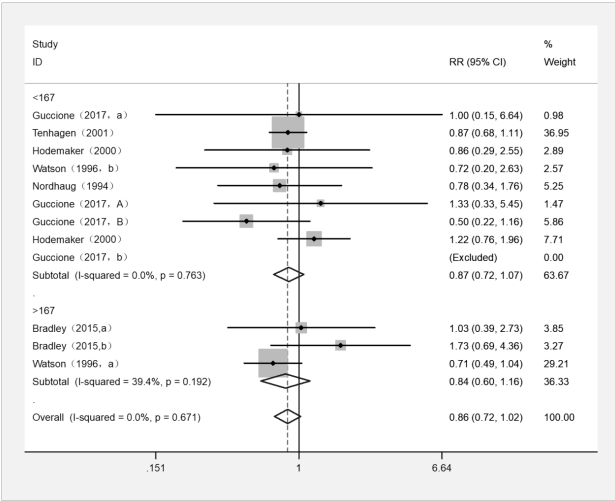

Figure 7. A forest plot of subgroup analysis assessing the influence of sample size

The result of sensitive analysis revealed that the conclusion of the efficacy of the *Staphylococcus aureus* vaccine was not stable. When two of twelve data sets were omitted, the pooling result converted to a positive conclusion with 95% CI excluding value 1 (Table 7). Contrarily, the conclusion of the efficacy of the *Escherichia coli* vaccine was stable for no conversion of the conclusion was detected (Table 8).

4. Discussion

The aim of this meta-analysis was to assess the efficacy of vaccine against mastitis caused by *Staphylococcus aureus* or *Escherichia coli* through pooling previous studies. The overall effect indicates that vaccination does not provide significant protection against bovine mastitis caused by *Staphylococcus aureus* or *Escherichia coli* with RR 0.86 at 95% CI $^{[0.72,1.02]}$ and 0.96 with at 95% CI $^{[0.81,1.12]}$ respectively, both showing the inclusion of the value 1 in the CI.

The results of quality assessment revealed half of 6 randomized controlled trials mentioned the "random" in the description, but failed to specify the method of random allocation, and compromised the quality of the study. One study allocated cows based on ear tags (odd number and even number was divided into two groups). Although this practice reduces the workload, it cannot be considered as an appropriate randomising method. The process of random allocation consists two main steps. The first step is the generation of random sequences. Multiple methods are available to achieve this goal, such as the random number table method and SAS. The generated random sequence is to be used as a random allocation scheme. The next step is to conceal allocation, and prevent personnel from consciously or unconsciously influencing the experiment outcome. Appropriate randomization can reduce the individual's impact on the results. For example, self-resolution is possible in mastitis, and will affect the evaluation of vaccine efficacy. However, this ability is different among individuals, and relevant factors include immune status and genetic resistance. Appropriate random allocation reduces the influence of these factors, and increases the accuracy of results. The results of a study without allocation concealment can be exaggerated by 30%-41% comparing to those with allocation concealment.

Some studies did not report whether the participants were blind to the treatment. Unlike human clinical trials, animals are inherently considered as blind participants under most circumstances, as a result, personnel are the only concern in this aspect. The blind method is implemented to ensure everyone involved in the experiment is unaware of the precise allocation plan, and preserves the impartiality of the evaluation. Pereira also pointed out in his systematic review that some trials may conceal true efficacy of vaccines due to the lack of double-blind measures [11]. This reminds the necessity of comprehensive design and detailed description regarding random allocation method

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in future studies.

It is also important to cover economic factor in future studies. The cost-profit analysis should cover the impact of withdraw period, productivity loss, and vaccination cost, for dairy industry is primarily profit driven. Ozsvari conducted a survey in a large-scale Hungarian dairy farm which initiated Startvac® application in 2010. The survey reported that the mastitis vaccination increased average annual profit by € 50.7 (decrease in loss-cost of vaccination) per cow between 2011 and 2014 [18]. Although Startvac® is not effective in reducing incidence, it may have a significant positive impact on reducing the quantity of discarded milk. Our meta-analysis suggested that the polyvalent inactive mastitis vaccine.

5. Conclusions

The results in this study revealed the efficacy of vaccine against mastitis caused by *Staphylococcus aureus* or *Escherichia coli*. The overall effect indicates that vaccination does not provide significant protection against bovine mastitis caused by *Staphylococcus aureus* or *Escherichia coli*.

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