Mastitis vaccination in dairy cattle: a meta-analysis of field case-control trials

Vacinação contra a mastite em vacas leiteiras: uma metanálise de ensaios clínicos de campo com controlo

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Resumo: A mastite é a primeira causa de perdas económicas no sector leiteiro. A eficácia das vacinas existentes contra a mastite é questionada por diversos autores. É o objectivo deste estudo, contribuir para o esclarecimento desta questão. Uma metanálise foi efectuada em 15 ensaios clínicos de campo, incluindo um total de 7941 vacas (4317 vaccinadas e 3624 usadas como controlo). Um modelo de efeitos aliatórios foi ajustado com uma significância de P<0,01. A unidade considerada foi o rácio (entre vacas vaccinadas e usadas como controlo) da razão entre vacas com mastite e normais. Este foi calculado como tendo um valor de 0,604 com um intervalo de confiança a 95% de [0,373; 0,979] depois da correção do enviezamento de publicação. Conclui-se que a vacinação para o controlo de mastites traz alguns resultados positivos, mas a vantagem económica do seu uso depende do balanço entre custos e benefícios. Como tal, as medidas preventivas tradicionais continuam a desempenhar um papel fundamental no controlo da mastite; a vacina contra a mastite pode ser utilizada como um complemento no combate à mastite, mas não como um substituto de todas as outras medidas preventivas.

Palavras-chave: mastite, mamite, vacas leiteiras, vacina, metanálise

Summary: Mastitis is the first cause of economic loss in the dairy sector. The efficacy of the existing vaccines against mastitis is questioned by several authors. It is the aim of this paper to shed some light on this question. A meta-analysis was conducted on 15 field research trials, which included a total of 7941 cows (4317 vaccinated and 3624 used as control). A random-effects model was adjusted and found significant (P<0,01). The unit used was the rate ratio between vaccinated cows and controls and was found to have a value of 0,604 with a 95% confidence interval of [0,373; 0,979] after correction for publication bias. It was concluded that vaccination to control mastitis has a slight advantage, but the economics of its use depends on the weighting between its cost and its benefit, and therefore traditional preventive measures still have an important role to play; Mastitis vaccines can be used as a complement of all the other preventive measures but not as a substitute of these.

Keywords: mastitis, dairy cow, vaccine, meta-analysis

Introduction

Mastitis is the first cause of economic loss by disease in dairy cattle in developed countries worldwide (Nielsen et al., 2010). These include losses in actual and potential production, shortening of productive life, losses in milk quality, medicine costs, costs of veterinary care (Hogeveen et al., 2011) and potential implications in the general health of the animal including reproductive performance (Dobson et al., 2008). Mastitis is characterised by an inflammation in one or more quarters of the udder and can have an infectious or a traumatic origin (Kudi et al., 2009). The most frequent agents of infection in dairy cattle are Staphylococcus aureus, Escherichia coli, Streptococcus uberis and Streptococcus dysgalactiae (Schukken and Kremer, 2001). Mastitis caused by the first of these agents is classified as contagious, and by the others environmental (Tyler and Ensminger, 2006).

The research into vaccines against Staphylococcus aureus started in the early 1990s (Michie, 2002), and with regards to mastitis, several vaccines have been tested and advances have been made (e.g. González et al., 1989) but there are doubts with regards to the efficacy of the commercially available vaccines (Leitner et al., 2003). There are several types of vaccines that have been tested, and with regards to S. aureus these include whole organism (cellular lysates, inactive and attenuated vaccines) and sub-units (toxins, surface proteins and polysaccharides) (Wallenmaq, 2010). Mono and polyvalent vaccines have been tested: S. aureus, E. coli, S. aureus / E. coli, S. aureus / Streptococcus / Streptococcus (e.g. Yoshida et al., 1984; Gonzalez et al., 1989; Giraud et al., 1997; March et al., 2010); these were tested in adult cows exposed before to the disease and in heifers not exposed to the disease (e.g. Calzolari et al., 1997, Moromoto et al., 2011); and also different inoculation protocols were followed using, none, one or two boosters (e.g. Nordhaug et al., 1994; Watson et al., 1996).

The difficulties with the development of a vaccine against mastitis were identified a long time ago; Mellen-
berger (1977) states that these difficulties are related to
the large number of different organisms involved in the
pathology and with the fact that when the pathogen re-
mains in the duct and alveoli as subclinical infection, it
is not attacked by the humoral antibodies, which happens
only in clinical mastitis cases, when the pathogens
penetrate the mucous membrane. Denis et al. (2011)
who has recently done a review of the state of the art in
mastitis vaccination, recognises the doubts regarding the
efficacy of the existing vaccines and points several po-
tential directions to follow in future developments; these
authors conclude that the design of an effective vaccine
is challenging, as the immune response in the mammary
gland is unique. Bovine mastitis is an evolving disease
and different pathogens have shown to have different
levels of importance in the development of the disease
through time (Bradley, 2002).

The aim of this study is to make a meta-analysis of exis-
ting clinical case-control trials, to bring to light important
knowledge regarding the efficacy of the mastitis vaccines.

Material and methods

The literature search was extensively performed with
the use of Google scholar for English, French, Spanish
and the Portuguese languages: http://scholar.google.
http://scholar.google.com.br, and also all the databases
assessed by the University of the West of England library
search engine of the Animal and Land sciences subject,
which include: CAB Abstracts, Cambridge Journals On-
line, Directory of Open Access Journals, EBSCO Host
Electronic Journals Service, Emerald, MEDLINE, Nexis,
PubMed, SAGE Journals Online, ScienceDirect, Taylor
& Francis, Willey Online Library, UWE e-journals at
OVID and Zetoc. Simple and multiple combinations of
the following key words were translated into the languag-
es considered and used in the search: mastitis, vaccine,
trial, case control, cow, dairy, cattle, and immunisation.
Inclusion and exclusion criteria were defined as follows:
Animals were considered to have mastitis when clinical
mastitis was identified; some trials report their results
considering number of quarters infected, which is im-
possible to convert into number of animals infected and
were, therefore, excluded; the minimum period of obser-
vations considered after vaccination was 3 months, but no
maximum was considered; experimental trials where the
animals were challenged with pathogens were not consid-
ered; only independent studies without conflict of inter-
ests were considered.

The outcome measure considered in the analysis was
the logarithm of the risk ratio (RR). The residual hetero-
genesis ($\tau^2$) was estimated with the restricted maximum-
likelihood estimator and found to be 0.576 with a Wald
95% confidence interval of $[0.249; 2.088]$. The homoge-
neity of the data was tested with the Cochran’s Q-test, and
was found to be significant ($Q=80.32$, df=14, $P<0.001$).

As the data was shown to be heterogeneous an initial
mixed effects model was used. The covariate modera-
tors used were: “absolute latitude of the location of the
trial” (to add for climatic effect); “year of publication of
the communication” (to add for genetic and technolog-
ical gains); and “duration” (to add for the temporal effect
due to different durations of the trials). The factor modera-
tors used were: “vaccine agent” (Staphylococcus aureus,
Escherichia coli, and multiple), “booster” (no booster, 1 or
2) and “exposed” (trials using heifers never exposed to
mastitis or undifferentiated age pre exposed). As none of
the moderators was found to be significant ($P>0.05$), a
random effects model was finally used in detriment of a
fixed effects model to add for the heterogeneity observed.

After the random effects model a radial plot was pro-
duced to analyse the sensitivity of the model by looking at
the source of heterogeneity and the extent of heterogene-
cy due to each study. The publication bias was evaluated
via a funnel plot and tested via a regression test (weighted
regression with multiplicative dispersion). The normality
assumption was evaluated via Q-Q normal plot, and as
seen in Figure 1, the assumption can be made once all the
studies fall in the pseudo confidence envelop.

The statistical analysis was performed with the free-
ware R CRAN for Windows® version 2.15.0. platform
x86_64-pc-mingw32/x64 (64-bit) (Comprehensive R
Archive Network , http://cran.r-project.org/). The specific
meta-analysis package “metaphor” (author: Wolfgang
Viechtbauer) was used.

Results

The meta-analysis was performed with 15 studies (Ta-
ble 1) that met the criteria set in the methodology. These
studies took place between 1984 and 2011 in several
places around the world. A total of 7941 cows were used

![Random-Effects Model](Image)
in the trials, 4317 were vaccinated and 3624 were used as controls.

The outcome measure considered in this study was the relative risk (RR) or risk ratio and is calculated as risk if vaccinated divided by the risk if not vaccinated. The random effects model adjusted was found to be significant ($P<0.01$) and the estimated log value is -0.644 [-0.207; -1.082] of the RR that after exponentiation gives the 0.50 [0.34; 0.81] value observed in the forest plot (Figure 2). The model is estimated to have a total amount of heterogeneity of $\tau^2 = 0.576 [0.249; 2.088]$, and the percentage of total variability due to heterogeneity is $I^2 = 91.7\% [82.9; 97.3]$. This heterogeneity was found to be significant after the Q test ($P<0.001$).

Figure 2 – Forest plot showing the results of the 15 studies examining the effectiveness of the mastitis vaccine. The relative risk ratio (RR) of mastitis infection favours the vaccine if lower than 1. The RR are presented with 95% confidence intervals, and are based on the adjusted random effects (RE) model.

<table>
<thead>
<tr>
<th>Author(s) and Year</th>
<th>Vaccinated # cows</th>
<th>Duration (month)</th>
<th>Vaccine agent</th>
<th># boost exposed</th>
<th>absolute latitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoshida et al., 1984</td>
<td>39</td>
<td>50</td>
<td>41</td>
<td>83</td>
<td>S.aureus</td>
</tr>
<tr>
<td>Gonzalez et al., 1989</td>
<td>6</td>
<td>227</td>
<td>29</td>
<td>169</td>
<td>E. coli</td>
</tr>
<tr>
<td>Cullor, 1991</td>
<td>7</td>
<td>212</td>
<td>16</td>
<td>229</td>
<td>E. coli</td>
</tr>
<tr>
<td>Nordhaug et al., 1994</td>
<td>0</td>
<td>58</td>
<td>6</td>
<td>44</td>
<td>S.aureus</td>
</tr>
<tr>
<td>McClure et al., 1994</td>
<td>25</td>
<td>640</td>
<td>78</td>
<td>646</td>
<td>S.aureus</td>
</tr>
<tr>
<td>Watson et al., 1996</td>
<td>45</td>
<td>866</td>
<td>67</td>
<td>843</td>
<td>S.aureus</td>
</tr>
<tr>
<td>Cullor et al., 1997</td>
<td>5</td>
<td>903</td>
<td>16</td>
<td>165</td>
<td>S.aureus</td>
</tr>
<tr>
<td>Giraud et al., 1997</td>
<td>9</td>
<td>71</td>
<td>18</td>
<td>22</td>
<td>Multi</td>
</tr>
<tr>
<td>Tenhagen et al., 2001</td>
<td>67</td>
<td>37</td>
<td>74</td>
<td>83</td>
<td>Multi</td>
</tr>
<tr>
<td>Leitner et al., 2003</td>
<td>3</td>
<td>226</td>
<td>6</td>
<td>218</td>
<td>Multi</td>
</tr>
<tr>
<td>Wilson et al., 2007</td>
<td>105</td>
<td>251</td>
<td>99</td>
<td>306</td>
<td>Multi</td>
</tr>
<tr>
<td>Iraian et al., 2007</td>
<td>29</td>
<td>111</td>
<td>20</td>
<td>107</td>
<td>S.aureus</td>
</tr>
<tr>
<td>Pol et al., 2008</td>
<td>34</td>
<td>133</td>
<td>50</td>
<td>120</td>
<td>Multi</td>
</tr>
<tr>
<td>Slobodanka et al., 2010</td>
<td>0</td>
<td>23</td>
<td>4</td>
<td>15</td>
<td>Multi</td>
</tr>
<tr>
<td>Morimoto et al., 2011</td>
<td>54</td>
<td>181</td>
<td>59</td>
<td>195</td>
<td>Multi</td>
</tr>
</tbody>
</table>

Figure 3 – Radial plot for the adjusted random effects model.

Figure 4 – Trim and fill funnel plot, highlighting 3 missing studies and a publication bias.

Table 1 – The 15 studies considered in this meta-analysis and the variables used as moderators. The number of vaccinated and control cows, together with the number of observed negative and positive to mastitis can be found in Figure 1.
The amount of overall heterogeneity due to each study is evaluated with the radial plot shown in Figure 3, and as can be observed all the studies considered fall in the confidence envelop, with a small exception. The RR of the different studies, having different precisions, show therefore to be consistent. This plot also shows some evidence of existence of publication bias, which can be even better observed through the trim and fill funnel plot (Figure 4), with the number of missing studies being estimated as 3, being 2 of them in the right hand bottom corner of the plot. This positioning gives evidence of small trials publication missing when results obtained contradict the expected positive effect of the vaccine. The rank correlation test for asymmetry (Kendall’s T) was found to be significant (P=0.05), and therefore a publication bias was found present. The new Overall RR after fill and trim for correction of asymmetry is 0.60 with a 95% CI of [0.37; 0.98].

Discussion

The history of mastitis vaccine is connected with the search for protection against the microorganisms causing it, and therefore started in the beginning of the last century (Michie, 2002; Wilson and González, 2003). The search of a bacterin to combat environmental pathogens (mainly Escherichia coli, but also Klebsiella spp, Enterobacter spp and Pseudomonas spp) is the aim of researchers since 1910, but not until the late 1980s was some efficacy reported (Wilson and González, 2003). The main antigen bacterin used for the production of anti-coliform vaccines is known as J5, but RE-17 mutant Salmonella typhimurium bacterin toxoid is also used. There are several of these vaccines available on the market, such as Pfizer Animal Health, Upjohn J-5 Bacterin®, Bayer Mastiguard®, Merial, J-Vac®; IM-MVAC Endovac-Dairy®, Novartis Animal Health J-5 Shield™.

The vaccination against Staphylococcus aureus has also been attempted from the beginning of last century, including attenuated, fixed or lysed organisms. Also in the 1960s polysaccharides from the capsule were used, and later in the 1970s enterotoxins were also used as antigenic components (Michie, 2002). More recently experimental DNA recombinant protein and recombinant protein alone vaccines are being tested (Denis et al., 2011). In present times one vaccine specific for S. aureus mastitis is available in the market, namely Boehringer Ingelheim, Lysigain® Somato-Staph®. Since the 1980s attempts have also been made to design a vaccine against Streptococcus uberis but this is still not available commercially (Denis et al., 2011).

HIPRA, Startvac®, is a vaccine commercially available and fights both E. coli and S. aureus through inactivated E. coli J5 and inactivated S. aureus (CP8) SP 140 strains.

From these vaccines, Startvac®, is the only one used in the UK and all the others are used in the USA. The meta-analysis did not identify any significant moderator, including the vaccine agent (including E. coli, S. aureus and multi agent), and therefore it was not found that a mixture of agents in the vaccine would improve its efficiency. Accordingly to the results of this study, all these types of vaccines will have the same degree of efficiency, and therefore the choice (where possible) should be done having in mind the application protocol and the final price for following the protocol.

The RR after fill and trim for correction of asymmetry was found to be 0.60 with a 95% CI of [0.37; 0.98], showing the exclusion of the value 1 in the CI and therefore an advantage on the use of vaccines against mastitis. Considering, however, the upper limit of the CI, full advantage is not completely evident when considering the cost of the vaccines.

The efficacy achieved so far with the vaccines existing in the market is still relatively low, and therefore ongoing discussions are taking place between scientists regarding the best strategy, while experimentation is also giving new clues about how to defeat mastitis (e.g. Leigh et al., 2010). Transgenic resistant cows have already been engineered, capable of expressing an antibacterial endopeptidase in their mammary glands, which specifically targets the cell wall of S. aureus (Rainard, 2005). Sordillo (2011) summarised some of the reviews done in the past decade and highlights some directions that research is taking to explore ways of improving vaccination efficiency: for example the need to develop protocols that enhance mucosal cellular immunity and trafficking of memory T cells to the mammary gland as a strategy to develop effective immunization; and the use of marker assisted technology to identify genes and chromosomal regions of interest.

DNA vaccination is a recent strategy, where plasmids are introduced in the animal to lead to the activation of both humoral and cellular immune responses to the antigen (Talbot and Lacasse, 2005).

Other moderators considered in the model were the absolute latitude to test an eventual effect of climate; year to test the evolution of the technology in the 27 years spread of trials used; the duration of the trial to consider the time effect, the number of boosters and the use of heifers or previously exposed groups of animals, but none of these was found to be significant. The moderator year is also not significant and therefore there is no evidence of developments in the efficacy of the vaccines used today when compared with those used 25 years ago.

This study shows an advantage on the use of vaccines to control mastitis that is not completely conclusive and agrees with the conclusions of the majority of independent authors writing about mastitis vaccination efficiency. A mastitis rate ratio for vaccinated versus control cows of 0.604 after correction for asymmetry, favours the vaccination, but with a 95%CI of [0.37; 0.979], this advantage is small. The advantage of the use of mastitis vaccines needs to be weighed between...
the costs of not using them with the costs of using them, but the use of the traditional preventive measures are still of fundamental importance. As indication Nielsen et al. (2010) has calculated the cost of a clinical mastitis case in Sweden in €278 when considering withdrawn of milk, lower prices due to high somatic cell counts, veterinary fees, drugs and increased risk due to recurrent cases; extra labour was not considered. The cost of a full vaccination programme with boosters and conferring protection for 1 year was reported to be between £7 and £9 by Balsom (2011).

Bibliography

tis-vaccine-another-weapon-in-the-toolbox.htm


