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# Systematic Review Evaluation of Vaccine Efficacy Against *Streptococcus agalactiae* in Fish

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**Abstract**

*Streptococcus agalactiae (group B* Lance field) has been considered an emerging pathogen, responsible for numerous outbreaks with high morbidity and mortality in fish. The search for protective vaccines against this pathogen has significantly intensified alongside the rapid expansion of aquaculture in the last decades. The present study aimed to systematically review the efficacy of vaccines described in literature for fish infections caused by *S. agalactiae* and, when possible, suggest which vaccines achieve the best and/or most promising results. An electronic search was performed using the PubMed and Web of Science data bases in January 2017. Only studies that tested vaccines *in vivo* and performed experimental and/or natural challenges in fish were selected. Studies with all types of vaccines were selected in order to determine the effects of different vaccine protocols on immune responses and protection during challenges. We selected nineteen papers for this review. The majority of studies showed a significant increase in immune response after vaccination and three studies did not evaluate immune response. This systematic review suggests that the use of new technologies, especially feed based vaccines, has achieved good results, which supports their use in the prevention and control of *S. agalactiae* infections in fish.

## INTRODUCTION

*Streptococcus agalactiae* (Lancefield group B; GBS) may infect several mammalian species and fish, mainly those cultivated in warm water. This pathogen may produce septicemia, granulomatous systemic inflammation and meningo encephalitis in infected fish, presenting various clinical signs such as skin hemorrhage, exopthalmia, ascites and erratic swimming [1]. Streptococcus's infection is the most serious disease for tilapia, and is responsible for huge economic losses in the tilapia farming industry worldwide every year [2,3].

GBS has recently been considered an emerging pathogen responsible for numerous outbreaks with high morbidity and mortality in wild fish, [4,5] in giant Queensland Grouper and some wild fish species in Australia [6]. There are various reports of infections in marine and freshwater fish species, both in the wild and in cultivation [7,8] particularly in tilapia [3-11]. Serotypes Ia and Ib are the most prevalent in seafood [12]. However, outbreaks worldwide show Ia, Ib and III as the most common serotype isolates from infected tilapia [4-13].

This devastating impact on tilapia production has led to increased antibiotics and other drug usage, which has raised serious concerns about drug consequences on the environment [14,15]. The least harmful disease control strategy is vaccination. As such, the search for protective vaccines against *S. agalactiae* has significantly intensified alongside the rapid expansion of tilapia production in the last decades.

The development of new vaccines and the improvement in vaccination techniques have helped control some infectious diseases in aquaculture, including streptococcus's outbreaks [16,17]. Different types of vaccines have been developed against GBS in fish [4-20]. However, the developed vaccines do not protect against all varieties of serotypes, leading to outbreaks with high mortality due to the prevalence of different serotypes and genetic profiles in the same region, suggesting the need to develop a polyvalent vaccine.

Despite the wide variety of vaccine studies for *S. agalactiae* in fish, there are observed absence of protocol standardization which may result in bias in the study results and dubious conclusion. Since different methodologies are applied in studies of this subject resulting in diverse reports with varying degrees of scientific evidence. Thus, literature revision studies are important and necessary because they use trusty methods to perform a thorough literature review, contributing to a clearer visualization of results and offering impartial suggestions regarding the best protocols to be employed and/or studied.

The aim of the present study was to systematically review the efficacy of vaccines described in literature for fish infections caused by *S. agalactiae* and, when possible, suggests which vaccines achieve the best and/or most promising results.

## MATERIALS AND METHODS

### Research strategy

We performed an electronic search in the PubMed databank (http://www.ncbi.nlm.nih.gov ) in January 2017 using the following keywords: ("fish") AND ("*Streptococcus agalactiae*") AND ("Vaccine"). Using a similar strategy, we performed a supplementary search in the Web of Science database (http://apps-webofknowledge.ez78.periodicos.capes.gov.br/WOS_GeneralSearch_input.do?product=WOS&search_mode=GeneralSearch&SID=3C5cPYseo7BxKPYltzK&preferencesSaved=) to confirm the findings and obtain additional studies.

### Selection of studies

The first criterion was that articles had to be written in English. We only selected studies where vaccines were tested *in vivo* and that performed experimental and/or natural challenges in fish. All types of vaccines were selected in order to determine the effect of different vaccine protocols on immune responses and protection during challenges. Articles that tested passive immunization were excluded. There was no restriction regarding neither the number of animals nor the date of the studies. We did not include literature reviews and articles where the main objective was not to test a vaccine.

### Data extraction

Two researchers conducted searches separately in order to select the articles that met the inclusion criteria. In case of divergence among selected papers, all criteria were reviewed and discussed. Table (1) summarizes the experimental design of each selected article.

**Quality criteria:** After selecting the articles and summarizing the data, we conducted a quality analysis as described in Table (2). Based on previous systematic reviews [21,22], we classified the parameters using scores, where score '2' means adequate, score '1' means partially adequate or unclear, and score '0' is inadequate. Parameters such as type of vaccine, route of administration, number of doses, adverse effects, bacterial load used in challenge, and whether the vaccine came from an autogenously strain, were not scored on the quality scale, but were taken into consideration since they were relevant for later discussions. The immune response was punctuated only in two scores, '0' for not significant stimulus of immune parameters and '1' for increasing of immunity. The scored parameters were as follows:

1. Sample number: Studies that used less than 10 animals per group were considered inadequate (score 0), studies using 10-50 animals per group were considered partially adequate (score 1), and those that used more than 50 animals per group were considered adequate (score 2).
2. Methodology used to detect negative animals for *S. agalactiae* prior to the experiment: Studies in which sampling was submitted to microbiological, histopathological (preferably using specific antibodies that recognize the pathogen) and/or molecular diagnosis (PCR techniques of target tissues, for example, brain, kidney and spleen) proving the individuals had not been previously exposed to the bacteria were classified with a score 2; studies that only evaluated the absence of clinical signs were classified with score 1; and studies that did not test the animals or did not evaluate clinical signs prior to the experiment were classified with score 0.
3. Bacterial strain used in the experimental challenge: Studies that tested the vaccine against homologous and heterologous bacterial strains were classified as adequate (score 2), studies that evaluated the natural incidence of *S. agalactiae* were classified as partially adequate (score 1), and those that only tested against homologous strains were classified as inadequate (score 0).
4. Effect of the vaccine on immune response: Studies in which the vaccinated group showed significant increase in immune response compared to the control group were classified with score 1, and studies in which no immunity parameter was measured or there was no significant difference between groups were classified with a score 0.
5. Degree of vaccine protection during the experimental challenge: Studies in which the vaccine showed relative percent survival (RPS) greater than 90% were classified with a score 2; studies with RPS between 50% and 90% were classified with score 1; and those where RPS was lower than 50% were classified with a score 0.
6. Testing of *S. agalactiae* carrier state after vaccination and experimental challenge: Studies that tested vaccinated animals who survived the bacterial challenge and did not detect *S. agalactiae* in organs and tissues with microbiological, molecular or histopathological tests were classified as adequate (score 2); studies that tested vaccinated animals who survived the bacterial challenge and detected *S. agalactiae* in organs and tissues (carrier state) were classified as partially adequate (score 1); and studies that did not test the vaccinated group or only tested animals that died after the bacterial challenge were classified as inadequate (score 0).
7. Randomization: Randomized experiments were considered adequate and classified with a score 2; studies in which the randomization process was unclear were classified with a score 1; and those with no randomization were considered inadequate and classified with a score 0.

The maximum total score possible was 13.

## RESULTS

The search in the PubMed databank resulted in 26 articles, and the Web of Science database resulted in 18 additional papers, totaling 44 articles. Of these, we excluded 25 that did not fit the aforementioned study criteria: 17 only studied the structure of bacterial proteins and/or characterization of strains and pathogenicity; three were literature reviews; one was not written in English; one tested a vaccine against other bacteria;

**Table 1**: Experiments regarding different protocols of *S. agalactiae* vaccines in fish.

| Authors | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Huang et al. (2014) [29] | 50 | Recombinant DNA vaccine using S. typhimurium as a vector | No | Clinical evaluation | Oral | 1, 2 and 3 | $10^8$ CFU/fish |
| Nur-Nazifah et al. (2014) [28] | 90 | Feed-based vaccine using a recombinant gene of cell wall surface anchor family protein of *S. agalactiae*; formalin-killed cells of *S. agalactiae* vaccine | No | Microbiological tests/ELISA | Oral | 2 | 2.27 x $10^9$ CFU/mL |
| Chen et al. (2012) [37] | 30 | Formalin-killed *S. agalactiae* vaccine of ten different strains (Va - Vj); Combination vaccines (Vg+Vb) and (Vg+Vd) | No | Microbiological tests | IP | 1 | identical genotype strains: ~2 x $10^8$ CFU/fish; mixture of strains: 1 x $10^8$ CFU/fish; combined vaccine: ~2 x $10^8$ CFU/fish |
| Pasnik, Evans and Klesius (2005)[38] | 45 | Extracellular products (ECP) and formalin-killed S. agalacitae whole cells | No | Not tested | IP | 1 | ~ 2.0 × $10^4$ CFU/fish |
| Pasnik et al. (2005) [18] | 45 | Extracellular products (ECP) and formalin-killed S. agalacitae whole cells stored at 4°C for 1 year | No | Not tested | IP | 1 | 1.7 x $10^4$ CFU/fish |
| Evans, Klesius and Shoemaker (2004) [39] | 20 (IP) and 65 (BI) | Formalin-killed cells and concentrated extracellular products of *S. agalactiae* | No | Not tested | IP and BI | 1 | 2.6 × $10^3$ to 1.7 × $10^6$ CFU/fish |
| Bei et al. (2014) [35] | 120 | Surface immunogenic protein (Sip) of *S. agalactiae* | No | Not tested | IM | 1 | 1.0 x $10^7$ CFU/ml |
| Pretto-Giordano et al. (2010) [24] | 41 - 50 | Inactivated *S. agalactiae* cells vaccine with formalin | No | Microbiological tests | IP | 1 and 2 | 3.0 x $10^6$ CFU/ml |
| He et al. (2014) [26] | 30 | Recombinant protein (Sip); G1: Sip; G2: Sip+aluminumhydroxide; G3: Sip+FICA | No | Not tested | IP | 2 | 1,5 x $10^8$ CFU / ml |
| Zhang et al. (2016) [40] | 20 | Recombinant protein (Sip) with gastric protection (immuneadjuvants and nanoparticles) | No | Not tested | Oral | 3 | $10^7$ CFU / ml |
| Wang et al. (2014) [41] | 25 | Live attenuated; recombinant proteins; both | No | Microbiological/ serological tests | IP | 2 | 4.26x $10^5$ CFU/fish |
| Li et al. (2015) [23] | 100 | Live attenuated | No | Microbiological tests | IP, BI and oral | IP: 1; BI: 1; Oral: 1, 2 or 3 | 1.0 x $10^6$ CFU/fish |
| Ismail et al. (2017) [27] | 900 | Bacterin | No | Microbiological tests | Oral | G4, 5, 6: 2; G7, 8, 9: 3 | - |
| Cai et al. (2016) [42] | 40 | Live attenuated | No | Microbiological tests | IP | 2 | 5,4 × $10^8$ CFU / ml |
| Ismail et al. (2016) [43] | 170 | Live attenuated | No | Microbiological/ serological tests | Oral | G1: 2; G2: 3 | 1.0 × $10^9$ CFU/ml |
| Liu et al. (2016) 44 | ~50 | Recombinant protein; live attenuated vaccine | No | Not tested | IP | 1 | 1x$10^9$ CFU/ml |
| Firdaus-Nawi1 et al. (2014) [25] | 72 | Live attenuated vaccine + FKB + Freund adjuvant or without adjuvant | No | Microbiological tests | Oral | 2 | 3.4 x $10^9$ CFU $mL^{-1}$ |
| Yi et al. (2014) [45] | 17 | Recombinant proteins | No | Microbiological tests and PCR | IP | 2 | 7.8 × $10^8$ $CFUml^{-1}$ |
| Noraini et al. (2013) [46] | 20 | Formalin-killed cells of *S. agalactiae* | No | Not tested | Spray | 3 | $10^9$ CFU/mL |

| Authors | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|
| Huang et al. (2014) [29] | Homologous | Yes (only after second dose) | 1 dose: 20%; 27%; 33%; 2 doses: 30%; 40%; 47%; 3 doses: 47%; 53%; 57% | Not tested | Yes |
| Nur-Nazifah et al. (2014) [28] | Homologous | Yes | 70% | *S. agalactiae* present in organs of dead fish (microbiological test and PCR) | UC |
| Chen et al. (2012) [37] | Homologous and heterologous | Not tested | identical genotype strains: 44.71% - 98.81%; mixture of strains: 13.33% - 60%; combined vaccine: 26.67% - 100% | Not tested | UC |

| | | | | | |
|---|---|---|---|---|---|
| Pasnik, Evans and Klesius (2005) [38] | Homologous | Yes | 47d: 67%; 90d: 62%; 180d: 49% | *S. agalactiae* present in organs of dead fish(microbiological test) | UC |
| Pasnik et al. (2005) [18] | Homologous | Yes | 29% | *S. agalactiae* present in organs of dead fish (microbiological test) | UC |
| Evans, Klesius and Shoemaker (2004) [39] | Homologous and heterologous | Not tested | IP vaccine: 70 - 80%; BI vaccine: 34% | *S. agalactiae* present in organs of dead fish(microbiological test) | Yes |
| Bei et al. (2014) [35] | Homologous | Yes | 90.62% | *S. agalactiae* isolated from organs of dead fish | Yes |
| Pretto-Giordano et al. (2010) [24] | Homologous | Not tested | T1 (1 dose) - 83.6%; T2 (2 doses) - 96.6% | T1 - *S. agalactiae* was isolated from organs; T2 - *S. agalactiae* was not isolated from organs (microbiological tests) | UC |
| He et al. (2014) [26] | Homologous | Yes | G1: 50%; G2: 55%; G3: 90%; G4: 0 | *S. agalactiae* present in organs (microbiological and histopathogical tests) | UC |
| Zhang et al. (2016) [40] | Homologous | Yes | 100% (nanoparticles group) | Not tested | UC |
| Wang et al. (2014) [41] | Homologous | Yes | 82,4% for G prot+vac | Not tested | UC |
| Li et al. (2015) [23] | Homologous | Yes (tested only in oral group) | IP: 96,88%; BI: 67,22%; oral: 71,81% | *S. agalactiae* present in organs (microbiological and molecular tests) | UC |
| Ismail et al. (2017) [27] | Natural infection | Yes | 2 doses: 65,3%; 3 doses:75,1% | Not tested | Yes |
| Cai et al. (2016) [42] | Homologous | Yes | 93.10% | *S. agalactiae* present in organs (microbiological test) | UC |
| Ismail et al. (2016) [43] | Homologous | Yes | G1: 45%; G2: 70% | Not tested | UC |
| Liu et al. (2016) 44 | Homologous | Yes | Protein: 72,5 to 82.5%; Inactivated vaccine: 54,5 to 62,5% | Not tested | UC |
| Firdaus-Nawi1 et al. (2014) [25] | Homologous | Yes | With adjuvant: 100%; without adjuvant: 50% | *S. agalactiae* present in organs (microbiological, molecular and histopathogical tests) | UC |
| Yi et al. (2014) [45] | Homologous | Yes | FbsA: 40,63%; α-enolase: 62,5% | Not tested | UC |
| Noraini et al. (2013) [46] | Homologous | Yes | IP challenge: 70%; BI challenge: 80% | *S. agalactiae* present in organs of dead fish(microbiological and molecular tests) | UC |

**Table 2:** Scores for evaluation criteria of selected articles.

| Authors | Number of animals per group[a] | Methodology used to detect negative animals prior the experiment[b] | Bacterial strain used in the experimental challenge[c] | Effect of vaccine on the immune response[d] | Degree of vaccine protection during the experimental challenge[e] | *S. agalactiae* carrier state post vaccination[f] | Type of experiment[g] | Total |
|---|---|---|---|---|---|---|---|---|
| Ismail et al. (2017) [27] | 2 | 2 | 1 | 1 | 1 | 0 | 2 | **9** |
| Li et al. (2015) [23] | 2 | 2 | 0 | 1 | 1 | 2 | 1 | **9** |
| Firdaus-Nawi1 et al. (2014) [25] | 2 | 2 | 0 | 1 | 2 | 1 | 1 | **9** |
| Chen et al. (2012) [37] | 1 | 2 | 2 | 0 | 2 | 0 | 1 | **8** |
| Nur-Nazifah et al. (2014) [28] | 2 | 2 | 0 | 1 | 1 | 0 | 1 | **7** |
| Cai et al. (2016) [42] | 1 | 2 | 0 | 1 | 2 | 0 | 1 | **7** |
| Huang et al. (2014) [29] | 1 | 1 | 0 | 1 | 2 | 0 | 2 | **7** |
| Bei et al. (2014) [35] | 2 | 0 | 0 | 1 | 2 | 0 | 2 | **7** |
| Ismail et al. (2016) [43] | 2 | 2 | 0 | 1 | 1 | 0 | 1 | **7** |
| Evans, Klesius and Shoemaker (2004) [39] | 2 | 0 | 2 | 0 | 1 | 0 | 2 | **7** |
| Pretto-Giordano et al. (2010) [24] | 1 | 2 | 0 | 0 | 1 | 2 | 1 | **7** |
| Wang et al. (2014) [41] | 1 | 2 | 0 | 1 | 1 | 0 | 1 | **6** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Yi et al. (2014) [45] | 1 | 2 | 0 | 1 | 1 | 0 | 1 | **6** |
| Liu et al. (2016) [44] | 1 | 2 | 0 | 1 | 1 | 0 | 1 | **6** |
| Zhang et al. (2016) [40] | 1 | 0 | 0 | 1 | 2 | 0 | 1 | **5** |
| He et al. (2014) [26] | 1 | 0 | 0 | 1 | 1 | 1 | 1 | **5** |
| Noraini et al. (2013) [46] | 1 | 0 | 0 | 1 | 1 | 0 | 1 | **4** |
| Pasnik, Evans and Klesius (2005) [38] | 1 | 0 | 0 | 1 | 1 | 0 | 1 | **4** |
| Pasnik et al. (2005) [18] | 1 | 0 | 0 | 1 | 0 | 0 | 1 | **4** |

a. Scores for the sample number were 0 (less than 10 animals/group), 1 (10-50 animals/group) and 2 (more than 50 animals/group).
b. Studies in which the animals were submitted to microbiological or molecular tests before experiment (score 2), studies that only performed a clinical evaluation (score 1), and those that did not test a sampling prior to the experiment (score 0).
c. Studies that tested the vaccine against homologous and heterologous bacterial strains (score 2) studies that evaluated the natural incidence of *S. agalactiae* (score 1), and those that only tested the vaccine against homologous bacterial strain (score 0).
d. Significant effect of vaccine in immune response (score 1), no immunity parameter was measured or no statistically significant difference in immune response (score 0).
e. Studies in which the vaccine showed RPS greater than 90% (score 2), studies that the RPS were between 50% to 90% (score 1), and those that the RPS were lower than 50% (score 0).
f. Studies that tested vaccinated animals who survived after the bacterial challenge and *S. agalactiae* was not detected in organs and tissues by microbiological, molecular or histopathological tests (score 2), studies that tested vaccinated animals who survived after the bacterial challenge and *S. agalactiae* was detected in organs and tissues (score 1), and studies that did not test the vaccinated groupor those that only tested animals that died after the bacterial challenge (score 0).
g. Randomized experiments (score 2), not clearly (score 1), without randomization (score 0).

**Table 3:** Checklist for experimental design and features of quality control for future studies of vaccines against *S. agalactiae* in fish.

| |
|---|
| 1- Control groups – the experiment should have a positive (not vaccinated and infected animals) and a negative control group. |
| 2- Acclimatization and testing the animals for the pathogen to confirm that they are negative before the beginning of the experiment by histopathology (wholw tissues), microbiology (at least brain, heart, spleen, kidney and liver) and molecular test in the same organs. |
| 3- Sample number – The number of animals may change according to the physical structure of where the study will be carried out, but it should have a minimum number for an adequate statistical analysis (>50). |
| 4- Number of doses – evaluate if one dose is enough for good protection or try to use the minimum number of doses as possible in order to cause less stress to the animals. |
| 5- Routes of administration – select the best route for each study design, and is preferable test more than one route to compare the protection degree. |
| 6- Immunity analysis – evaluate if the vaccine causes significant increase in the immune response in vaccinated fish. |
| 7- Use of heterologous challenge with different strains (preferable using different serotypes and genotypes strains), to evaluate the protection against different lineages. |
| 8- Verify the carrier status of the pathogen after the experimental challenge through microbiological, histopathological and molecular tests. |

one did not carry out an *in vivo* experimental challenge; and one was a toxicological study. Lastly, one of the selected articles was retracted and we decided not include it in the review.

Thus, a total of 19 articles were selected for the present review (Table 1). The number of animals in these studies ranged from 17 to 900 fish per group. Regarding the type of vaccine, seven studies tested formalin-killed cells vaccine using one or more bacterial strains, and seven tested recombinant vaccines using DNA, genes or proteins of *S. agalactiae*. In six articles, live attenuated vaccines were tested; and three papers used extracellular products of *S. agalacitae* as a vaccine. Only one study tested bacteria in (killed vaccine or inactivated bacteria), and one of the articles tested a vaccine stored at 4°C for one year. Some studies tested more than one type of vaccine and/or a combination of them.

In 11 studies, the animals were tested prior to the experiment using microbiological/molecular tests or clinical evaluation. None of the studies used an autogenous vaccine. Sixteen studies tested a vaccine against homologous bacterial strains, while two studies tested against homologous and heterologous strains, and only one study evaluated the natural incidence of *S. agalactiae*. The bacterial dose used in the experimental challenges ranged from 1000 to $3.4x10^9$ colony-forming units (CFU).

Regarding the route of administration, nine studies tested intraperitoneal (IP) vaccines, six tested oral vaccines, one tested an intramuscular (IM) vaccine, and one tested a spray vaccine. Some studies tested vaccines using more than one route: one article tested IP and bath immersion (BI) vaccine, and another tested IP, BI and oral vaccines.

In six studies the tested vaccine presented a degree of protection higher than 90%, 12 studies presented an intermediate protection (50 to 90%), and only one study presented a low protection (lower than 50%). In studies that tested different types of vaccines or different doses, we considered the one that presented the highest protection. Among the studies that presented the best protection, two studies tested recombinant vaccines, two tested live attenuated vaccines, one tested sip vaccine, and one tested formalin killed vaccine.

In two articles [23,24] *S. agalactiae* was not detected in organs

and tissues of vaccinated animals who survived the bacterial challenge with microbiological, molecular or histopathological tests. In two of the articles [25,26] *S. agalactiae* was detected in vaccinated animals who survived the bacterial challenge, and 15 studies did not test vaccinated animals or tested only fish that died after the bacterial challenge.

In 16 studies, we observed a significant increase in immune response after vaccination and three studies did not evaluate immune response. Four studies were randomized and the remaining articles did not provide information regarding randomization. Only one study observed that the vaccine did not present adverse effects, the remaining articles made no mention of adverse effects.

## DISCUSSION

Systematic reviews provide a summary of various studies in a single document, simplifying the comparison of results and offering impartial suggestions about the best protocols to apply and/or study [21]. We performed this systematic review on protective effects of vaccines for *S. agalactiae* infections in fish to determine what types of vaccines and protocols show the most promising results. One limitation of this study we would like to emphasize is that the keywords used in the searches may be different from those used by other authors in their studies, which means that some articles published in the same field may not have been accessed. Another limitation is that, although the selected articles belonged to the same subject, the authors used different methodologies, which made it difficult to analyze and organize the data.

The present review aimed to find the study that presented the best vaccination protocol, regarding not only the degree of vaccine protection, but also other criteria that interfere in the applicability of the vaccine, such as route of administration and number of doses. Thus, studies that had the best scores were not necessarily the ones with the best protocols. Three articles received a score of 9 (best score), two [23-25] presented a degree of vaccine protection higher than 90%, and the third [27] showed intermediate degree of vaccine protection (50-90%). The number of doses in these studies ranged from one to three doses. The authors of the first study used bacterin vaccine [27] and the other two studies used live attenuated vaccines [23-25]. All three studies tested oral vaccines, and one of them [23] tested IP and BI vaccines as well as the oral vaccine, with the IP vaccine showing a greater protection in comparison to the others routes of administration.

Oral feed vaccination is the preferred route since it consumes less time, results in less labor work, has a low cost and is easier to perform by farmers [28]. One disadvantage of oral vaccines is that the antigen needs to be protected against inactivation or digestion by nucleases during its passage through the stomach and anterior gut to be effective. For this reason, the use of entero bacteria as a vehicle for oral vaccination has been widely used and studied in animals as an alternative [29]. However this study did not evaluated previous status of GBS infection and carrier state of GBS in experimental fish and without these factors it is not possible evaluate the real effectiveness of vaccination

The use of adjuvant is known to stimulate immune reactions in vertebrates, including fish. When combined with vaccines they may increase the protection level following exposure to pathogens [30]. Authors tested a live attenuated vaccine with and without adjuvant; the group that used adjuvant showed a protection of 100%, whereas the group without adjuvant presented only 50% protection [25].The adjuvant in itself induces a general and high stimulation of B-cells, which leads to a high production of IgM [30]. However, the study with 100% protection did not evaluate the carrier state of GBS.

Only one study tested a killed vaccine (bacterin) against a natural outbreak of *S. agalactiae* [27], showing a degree of protection between 65 and 75%. In this study, as well as *S. agalactiae*, other pathogenic bacteria were isolated from kidney samples of all tested groups, such as *Plesiomonas shigelloides, Enterobacter cloacae, Edward siella spp.* and *Staphylococcus spp*. Co infections are very common in nature and they occur when hosts are infected by two or more different pathogenic agents causing simultaneous infections. Co infections are ordinary in fish and may increase the impact of disease outbreak. The susceptibility of fish to different pathogens may be altered during mixed infections, which may result in sudden outbreaks in fish [31]. Fish production under high density growth conditions requires effective vaccines to control persistent and emerging diseases. It is also a known fact that vaccines may have a significant impact on reducing the use of antibiotics [32].

Few studies evaluated the presence of carrier status after experimental challenge [23-26]. Additionally, fish treated with oxytetracyclin after infection by *S. agalactiae* presented carrier status [33], suggesting that the same event may occurs in vaccinated fish since the vaccine does not provide complete protection against bacterial infection. Thereby, this feature is pivotal to vaccine studies.

The occurrence of *Streptococcus* infections increases when fish become stressed due to inadequate water temperature, low oxygen and high nitrite levels [34].Some authors evaluated the water quality and suggested that temperature levels may increase fish susceptibility to infectious diseases [27]. This may be observed as immune deregulation initiating due to heat stress response [28].

Seven articles tested recombinant vaccines suggesting that new technologies should be further investigated using immunogenic proteins and associating two or more genes in order to produce a vaccine of greater efficacy [10]. For example, the effectiveness of these technologies (with a high score in the quality criteria) was demonstrated by researchers who used recombinant DNA vaccines and an oral route of administration [28,29]. Since *S. agalactiae* has many different serotypes, the development of a vaccine that provides heterologous protection has been a major concern. Recently more attention has been focused on the development of vaccines based on genetically conserved antigens present in all *S. agalactiae* serotypes [36,37].

Finally, we propose a checklist for experimental design and features of quality control that might be used for vaccine development against *S. agalactiae*, and it could be useful to avoid basic faults observed in the articles used in this review. However, this checklist is only a guide, and certainly, some points suggested

should be changed according to specific objectives of each study (Table 3).

## CONCLUSION

Vaccines that use new technologies especially feed based vaccines; have achieved good results, which support their use in the prevention and control of *S. agalactiae* infections in fish. Nevertheless, different methodologies, lack of standardization and in some cases the absence of adequate scientific criteria hinder the evaluation of the effectiveness of these vaccination protocols.

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