Efficacy of *Brucella abortus* S19 and RB51 vaccine strains: a systematic review and meta-analysis

Short running tittle: Efficacy of *Brucella abortus* vaccines

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Abstract

Bovine brucellosis is a worldwide zoonotic disease, *Brucella abortus* S19 and RB51 being the vaccine strains most widely used for its control worldwide. This systematic review and meta-analysis aimed to recalculate the efficacy of these two vaccine strains, and to discuss the main variables associated with controlled trials to evaluate bovine brucellosis vaccines efficacy. The most used vaccine strain was S19, at the dose of 10^{10} colony forming units (CFU), followed by the vaccine strain RB51, mainly at 10^{10} CFU. The most used challenge strain was *B. abortus* 2308, at the dose of 10^7 CFU by intraconjunctival route. For the meta-analysis, the trials were grouped according to the vaccine strain and dose to recalculate protection against abortion (four groups) or infection (five groups), using pooled risk ratio (RR) and vaccine efficacy (VE). In the meta-analysis for protection against abortion (n = 15 trials), S19 vaccine at 10^9 CFU exhibited the

highest protection rate (RR = 0.25, 95% CI: 0.12 to 0.52; VE = 75.09%, 95% CI: 48.08 – 88.05), followed by RB51 10^{10} (RR = 0.31, 95% CI: 0.16 to 0.61; VE = 69.25%, 95% CI: 39.48 – 84.38). In the meta-analysis for protection against infection (n = 23 trials), only two subgroups exhibited significant protection: S19 at 10^9 CFU (RR = 0.28, 95% CI: 0.14 to 0.55; VE = 72.03%, 95% CI: 57.70 – 81.50) and RB51 at 10^{10} CFU dose (RR = 0.43, 95% CI: 0.22 to 0.84; VE = 57.05%, 95% CI: 30.90 – 73.30). In conclusion, our results suggest that the dose of 10^9 CFU for S19 and 10^{10} CFU for RB51 are the most suitable for the prevention of abortion and infection caused by *B. abortus*.

Keywords: bovine brucellosis, vaccination, abortion, infection, protection.

1- Introduction

Bovine brucellosis is mainly caused by *Brucella abortus,* and even though the disease has been eradicated from domestic animals in several countries from Europe, North America and Oceania, it is still prevalent in Latin America, Africa and Asia (Zhang et al., 2018). Brucellosis is highly contagious among animals, since a low infectious load is necessary to the transmission by aerosols (Carvalho Neta et al., 2010). The disease tends to spread quickly within the herd, causing decrease in milk and meat production, disposal of infected animals, besides reproductive signs, as abortions, stillbirth and infertility, which validated the use of control and prevention measures, especially vaccination (Olsen & Stoffregen, 2005; Dorneles et al., 2017). Associated with its great importance for animal health, brucellosis is classified by World Health Organization (WHO) as a neglected disease (WHO, 2015) and, in 2018, it was reported as the most prevalent zoonosis worldwide (Cross et al., 2019).

Vaccination is the central measure to control bovine brucellosis and the most used vaccines strains are *B. abortus* S19 and RB51 (Dorneles et al., 2015a). For female calves, the World Organisation for Animal Health (OIE) (OIE, 2016) recommends the use of S19 at a dose of 5-10 x 10^{10} colony forming units (CFU) (3 to 6 months of age) and RB51 at a dose of 1-3.4 x 10^{10} CFU (4 to 12 months of age). Moreover, S19 can also be used by the intraconjuntival route in heifers and cows of any age with one or two doses of 5×10^9 viable organisms (Nicoletti, 1990; OIE, 2016). This vaccine, used since 1941, is a smooth attenuated *B. abortus* biovar 1 strain that induces an antibody response that cannot be distinguished from the one induced by the infection (Manthei, 1959; OIE, 2016). The RB51 vaccine was developed in 1982 and it is a rough rifampicin-resistant *B. abortus* biovar 1 strain that does not express the O-side chain lipopolysaccharide (LPS) on its membrane, thereby, this vaccine does not induce antibodies detected by routine serological tests (Olsen & Stoffregen, 2005). For this reason, S19 vaccination is recommended for animals from 3 to 8 months of age (antibodies will decrease and will not interfere with routine serological tests about 4-6 months from vaccination), whereas RB51 vaccination can be performed in any heifer at any time from 3 months of age (Olsen & Stoffregen, 2005; Dorneles et al., 2015a)

Experiments designed to evaluate *B. abortus* vaccines involving bovine experimental infections, have a high cost (purchase and maintenance of animals for long periods, serological and bacteriological tests, need of specialized human resources, etc), are time consuming (around 24 months) and require biosafety level 3 facilities for large animals. Furthermore, there are also ethical issues related to the use of animals for experimentation, and the number of animals needed for the results to be statistically significant is generally high.

Albeit several studies have shown that S19 and RB51 vaccination protects about 65-75% of vaccinated animals against abortion and infection (Manthei et al., 1952; Nicoletti, 1990; Olsen, 2000a; Olsen & Stoffregen, 2005; Poester et al., 2006), the efficacy of bovine brucellosis vaccination is a subject that deserves more investigation due to it is crucial importance to animal and public health. Indeed, in the previous studies on brucellosis vaccine efficacy there is still some discussions on the ideal vaccine dose and route, the challenge dose, the stage of pregnancy at challenge, among other factors that need to be assessed to design optimized brucellosis vaccine assessment assays, which can be used for testing new vaccine candidates. Moreover, and even more significant, the calculation of vaccine efficacy in most of published studies is inappropriate, as it does not take into account results in control groups. Altogether these arguments reinforce the importance of conducting systematic reviews of the scientific literature in this field, to reach some consensus (on doses, strain, routes, etc.) and to recalculate the efficacy of vaccine strains at recommended doses.

In this context, a systematic review can help to assess the importance of different variables for both S19 and RB51 vaccines, while a meta-analysis can be used to recalculate vaccine efficacy, using a more robust number of animals. All the reproductive clinical signs reported in the articles as stillbirth, live-weak or premature calves and abortion, were considered for the meta-analysis as abortion *lato sensu*. Thus, the aims of this systematic review were to discuss the main variables associated with the experimental studies used to determine the efficacy of S19 and RB51, as well as to perform a meta-analysis to recalculate the S19 and RB51 efficacy (defined either as protection against abortion *lato sensu* or protection against *B. abortus* infection) for cattle.

Commented [JG1]: This is important to capture the meaning of "abortion"

Commented [ED2R1]: I agree, however don't you think that should be better at M&M section?

Commented [JG3R1]: Ok as written now.

2- Material and methods

The guidelines of PRISMA statement (Preferred Reported Items for Systematic Reviews and Meta-Analysis) were adopted in this review (Supplementary Table S1).

2.1- Strategy of search and selection of the studies

The search was conducted on July 26th, 2019. The selected keywords were investigated within all the sections from papers (title, abstract and full-text) in the following databases: CABI, Cochrane, PubMed, Scielo, Science Direct, Scopus and Web of Science. Briefly, the PICOT (population, intervention, comparison, outcome and time) involved cattle, *B. abortus* S19 and RB51 vaccine strains, vaccination against brucellosis, challenge, immunity, efficacy and protection, without restrictions regarding the time when the studies were published. An overview of the search terms is shown in the Supplementary Table S2.

In the first stage of selection, the studies were selected based of their tittles (MMO and CRP). Then, two reviewers (MMO and CRP), independently, evaluated each abstract. Subsequently, full-text of the selected papers based on the abstract were evaluated in terms of their relevance and by means of inclusion/exclusion criteria. When the two reviewers disagreed, a third one (EMSD) was responsible for the final decision. Further, the referential lists of the selected papers were reviewed to find pertinent studies not identified during the initial search.

2.2- Inclusion and exclusion criteria

The following characteristics were considered for the inclusion of articles: (*i*) approach on *B. abortus* vaccination using S19 or RB51, (*ii*) challenge of cattle with *B. abortus* virulent strain and (*iii*) evaluation of vaccine efficacy by means of a clinical trial. Articles focusing on (*i*) other *Brucella* species, (*ii*) genetics, immunology, microbiology, or drug therapy, (*iii*) vaccine efficacy assessed by field studies or (*iv*) written in languages other than English, Spanish, French and

120 Portuguese were excluded. Full inclusion and exclusion criteria are shown in the Supplementary
121 Table S3.
122
123 *2.3- Type of studies*
124 Original experimental studies were included. Papers as cohort, case-control, cross
125 sectional, case series, case reports and reviews were excluded.
126
127 *2.4- Data extraction*
128 Data were extracted from papers by one of the reviewers (MMO) and then checked for
129 accuracy by another reviewer (EMSD). Disagreements regarding data extraction among
130 reviewers were solved by consensus. Extracted data included: first author, year of the publication,
131 geographic location, breed of animals, number of animals used, number of animals per group,
132 animals age at vaccination, animals age at pregnancy, vaccine strain(s), vaccine dose, vaccine
133 route, number of vaccinations, interval between vaccination(s) and challenge, pregnancy stage at
134 challenge, challenge strain, challenge dose, challenge route, data on protection against clinical
135 signs (abortion, stillbirth and weak calves), data on protection against infection (maternal and
136 fetal bacteriology), vaccine clearance and serologic response post vaccination and post challenge.
137 Experimental studies without control groups or that did not report pregnancy stage or age of
138 animals at challenge, vaccine dose, strain, and route, challenge dose, strain, and route, and either
139 clinical protection (reproductive signs) or infection protection were excluded.
140
141 *2.5- Meta-analysis*
142 The trials were grouped for the meta-analysis based on their similarity regarding vaccine
143 strain and dose, and stage of pregnancy at challenge. Only data from single vaccination were
144 included in the meta-analysis. Moreover, for all meta-analysis groups, vaccination was performed
145 by subcutaneous route, the challenge dose was close to or 1 x 10^7 CFU and all animals were
146 exposed to virulent *B. abortus* between 4 and 7 months of pregnancy (Manthei, 1959; Nicoletti,

Commented [JG4]: L211-218: you mention trials with boost or revaccination: is this a contradiction?
L222: more, what about studies in which both vaccines were used?
A little bit confusing...

Commented [ED5R4]: For the systematic review we have included all paper that fitted all the criteria defined in the supplementary table S3 (29 papers and 51 trials). However, just some of them (17 papers) were used in the meta-analysis, as we used only those that vaccinated animals once, subcutaneously at the middle of pregnancy using a challenge dose close to 10^7. We described the methodology used by all 29 papers, but we used only part of the to recalculate the vaccines efficacy.

Commented [JG6R4]: My mistake. I added L217-218 "not include in the meta-analysis", to re-emphasize (up to you, of course)

1990; Moriyón et al., 2004). Two outcomes were considered for meta-analysis: protection against reproductive clinical signs and protection against infection. All the reproductive clinical signs reported in the articles as stillbirth, live-weak or premature calves and abortion, were considered for the meta-analysis as abortion *lato sensu*. The Mantel-Haenszel method (Dohoo et al., 2009) was used to calculate the effect estimate. When random-effects model was used, the variance of the distribution of true effect sizes, τ^2, was estimate by the Hartung-Knapp-Sidik-Jonkman method (Sidik & Jonkman, 2007) and the Hartung and Knapp method was used to adjust test statistics and confidence intervals (Hartung & Knapp, 2001) The homogeneity among the studies within a subgroup was evaluated by Cochrane's Q-statistic, Higgin's & Thompson's I^2 and τ^2 (Harrer et al., 2019). If the test for heterogeneity was significant, the random-effects within, fixed-effects between model was used, otherwise the fixed-effects (plural) model was used (Borenstein & Higgins, 2013). Treatment arm continuity correction in studies with zero cell frequencies (Sweeting et al., 2004) were used in all models. Test for subgroups differences was done by the Cochrane's Q-statistic (Harrer et al., 2019). The pooled risk ratios (RR) and 95% confidence intervals (95% IC) were obtained for each vaccine subgroup (strain/dose). Vaccine efficacy (VE) was estimated in the form of an attributable fraction [(1 - RR)*100], where the vaccination is the exposure or risk factor positive, and its 95% confidence interval was calculated by the substitution method (Daly, 1998). It can be interpreted as the fraction of the cases (abortion *lato sensu* or infection) under exposure (vaccination) that could be prevented by exposure (vaccination) (Dohoo et al., 2009). Vaccine strain and dose (meta-analysis groups) that exhibited a RR < 1 and in which the confidence interval did not include the null value (RR = 1) were considered effective. The meta-analyses were performed with R statistical software version 4.0.5 (Team, 2021), using the packages meta (Balduzzi et al., 2019) and dmetar (Harrer et al., 2019)

Commented [JG7]: This is important to capture the meaning of "abortion lato sensu". I am wondering if this could not be placed in the introduction.

Commented [ED8R7]: Don't you think that we could keep this Only here at M&M section? I thought it a little bit "strange" in the introduction. Would you agree?

Commented [JG9R7]: Agreed.

Commented [JG10]: No need to define? Up to you.

3- Results

3.1- Selected studies

The literature review included papers published between 1952 and 2016. The search strategy adopted identified a total of 4738 papers; 1246 duplicates were excluded, and 157 full-

texts were assessed for eligibility. Subsequently, 43 were evaluated by quality level assessment and 29 were included for data synthesis appraisal, after a thorough review (Figure 1). The main reasons for exclusion of these 14 paper for quality were absence of detailed methodology, including insufficient data about challenge (n = 4) (Mc Diarmid, 1957; Hendricks & Ray, 1970; Corner & Alton, 1981; Baldi et al., 1996), insufficient data about vaccination (n = 6) (Mc Diarmid, 1957; Hendricks & Ray, 1970; Worthington et al., 1974; Heck et al., 1982; Butler et al., 1986; Hall et al., 1988), data also presented elsewhere (n = 1) (Crawford et al., 1991), absence of control group (n = 2) (García-Carrillo, 1980; Crawford et al., 1988), and insufficient data on interest outcomes (n = 3) (Sutherland et al., 1982; Sutherland, 1983; Olsen et al., 1997). As a study can comprise multiple trials, an entire manuscript was referred to as a ‘‘study’’, whereas a single vaccine-to-control comparison in a study was referred to as a ‘‘trial’’. From the 29 selected studies, 13 [44.83% (13/29)] conducted a single trial, while 16 [55.17% (16/29)] studies comprised at least 2 trials, reaching a total of 51 trials assessed (Table 1). Assessment on the year of publication showed that 15 of the 29 papers [51.72% (15/29)] dated from before 1990 and 14 [48.27% (14/29)] were from years after this date until 2016.

3.2- Protection assay experimental designs

Cattle breed most used in the bovine brucellosis vaccines protection studies was crossbreed [24.13% (7/29)], followed by Hereford [17.24% (5/29)] and Jersey [17.24% (5/29)], Holstein [10.34% (3/29)], Kazakh [6.89% (2/29)], Criollo [3.45% (1/29)] and Limousine [3.45% (1/29)]. One study [3.45% (1/29)] (Manthei et al., 1952) used both Holstein and Jersey breeds, while four studies [13.79% (4/29)] did not provide information on the breed used (Supplementary Table S4). Holstein-Friesian and Frisonne breeds were grouped as Holstein, since both are considered variations of that breed (Porter et al., 2016).

The total number of animals used in the studies varied from 5 to 109, with an average of 24.89 (± 16.96) and a median of 20 [interquartile range (IQR) = 19]. The average number of vaccinated animals per group was 15.56 (±11.15) with a median of 12 (IQR = 8), whereas in control group the average number of animals was 11.74 (± 8.52) and the median 10 (IQR = 6).

Among those studies that performed the challenge of pregnant animals (n = 24), the pregnancy of the heifers was achieved by natural mating in most of the studies [62.50% (15/24)], 25.00% (6/24) used artificial insemination, 4.16% (1/24) both and 8.33% (2/24) did not provide this information (Supplementary Table S4). From the 51 trials assessed, 84.31% (43/51) performed the challenge in pregnant cows and 15.68% (8/51) the challenge in non-pregnant animals. Among those trials that challenged pregnant animals, 6 [11.76% (6/51)] also performed vaccination during pregnancy (Alton et al., 1980; Poester et al., 2006; Tabynov et al., 2014a; Tabynov et al., 2016). Single dose of bovine brucellosis vaccine was tested by 86.27% (44/51) of the trials, whereas 7 trials [13.72% (7/51)] performed booster vaccination (Table 1 and Supplementary Table S5). In six trials [11.76% (6/51)] a second dose of S19 was performed, using 10^7 CFU (Wyckoff et al., 2005) or 10^9 CFU (Plommet & Fensterbank, 1976; Fensterbank & Plommet, 1979; Plackett et al., 1980), by subcutaneous or intraconjunctival route. Only one trial [1.96% (1/51)] performed a second dose of RB51, using 10^9 CFU by subcutaneous route (Olsen, 2000b). Figures 2 and 3 show the main information on experimental design of the trials used to assess the efficacy of S19 and RB51. Detailed information about booster vaccination, not include in the meta-analysis, is shown in Supplementary Table S5.

3.3- Vaccine strain, dose and route

Regarding the vaccine strain used, 20 of the 29 selected studies (68.96%) used only S19, 5 [17.24% (5/29)] tested only RB51, while both vaccine strains were assessed in 4 studies [13.79% (4/29)]. Considering the 51 trials, 39 tested S19 [76.47% (39/51)] and 12 RB51 [23.52% (12/51)] (Table 1). The S19 vaccine dose ranged from 1 x 10^7 to 1.15 x 10^{11} CFU. Logarithmic grouping of tested S19 vaccine doses showed that 10^{10} CFU [51.28% (20/39)] was the most tested dose among all trials, followed by 10^9 CFU ([20.51% (8/39)], 10^8 CFU [10.25% (4/39)], 10^7 CFU [7.69% (3/39)], and 10^{11} CFU [2.56% (1/39)] (Figure 3). The remaining trials that tested S19 performed a booster vaccination using different doses at first and second vaccination. One trial [2.56% (1/39)] used 1.15 x 10^{11} CFU for the first vaccination and 5.7 x 10^9 CFU for the second one (Fensterbank & Plommet, 1979), and two [5.12% (2/39)] performed the first vaccination

using 9 x 10^{10} CFU and the booster with 4.5-5.0 x 10^{9} CFU (Plommet & Fensterbank, 1976; Plackett et al., 1980). For RB51, the vaccine dose ranged from 1 x 10^{9} to 3.4 x 10^{10} CFU, being 10^{10} CFU the dose assessed in 66.67% (8/12) of the trials, whereas 33.33% (4/12) used 10^{9} CFU (Table 1, Figures 2 and 3). Booster vaccination using RB51 using both times 1 x 10^{9} CFU was assessed in one trial [8.33% (1/12) (Olsen, 2000b).

The vaccine route used was mostly subcutaneous [84.31% (43/51)] for both vaccine strains, 3.92% of the trials (2/51) performed intraconjunctival vaccination (S19) (Plommet & Fensterbank, 1976; Fensterbank & Plommet, 1979), 1.96% (1/51) used oral route (RB51) (Elzer et al., 1998), 1.96% (1/51) the intradermal (S19) route (Manthei et al., 1952), and 1.96% (1/51), the intracaudal (S19) route (Buddle, 1948) (Table 1 and Figure 2). Three trials [5.88% (3/51)] used two different routes of vaccination, subcutaneous at the first vaccination and intraconjunctival for booster (Plommet & Fensterbank, 1976; Fensterbank & Plommet, 1979; Plackett et al., 1980). The vaccine dose volume inoculated for S19 vaccination was mostly 2 mL [33.33% (13/39)], however some trials also used 1 mL [10.25% (4/39)], 5 mL [5.12% (2/39)], 0.1 mL [2.56% (1/39)], 0.2 mL [2.56% (1/39)] or 4 mL [2.56% (1/39)]. Three trials [7.69% (3/39)] used two different vaccine dose volumes in prime and booster vaccinations (Manthei et al., 1952; Plommet & Fensterbank, 1976; Plackett et al., 1980) and 14 trials [35.89% (14/39)] did not inform the vaccination volume used. For RB51 vaccination, half of the trials used 2 mL [50% (6/12)], 25% (3/12) used 4 mL, and 25% (3/12) did not provide this information (Supplementary Table S4).

3.4- Age at vaccination and age or pregnancy stage at challenge

In 56.86% (29/51) of the trials, vaccination was performed in calves up to 12 months of age, whereas 33.33% (17/51) used animals from 12 to 24 months of age (Table 1 and Figure 2). Six trials [11.76% (6/51)] vaccinated pregnant animals, at 2 to 4 months of pregnancy. From these trials, one (Poester et al., 2006) vaccinated only part of the animals (8/20) at early pregnancy (60^{th} day of gestation) and another (Alton et al., 1980) vaccinated cows during their second pregnancy (n = 9).

The efficacy of vaccines against bovine brucellosis is normally assessed by challenging pregnant heifers with virulent *B. abortus*. However, 15.68% (8/51) of the selected trials challenged non-pregnant animals, in an average of 6 (± 0.83) months after vaccination (Figure 2). Among those trials that challenge animals during pregnancy [84.31% (43/51)], the stage of pregnancy at challenge range from 1.5 to 7.5 months, being more frequent among 4 to 7 months [76.74% (33/43)]. One study challenged the animals only once at one of five different pregnancy stages: up to 3 months, from 3 to 4 months, from 4 to 5 months, from 5 to 6 months, and over 6 months of pregnancy (Crawford et al., 1990).

3.5- Challenge strains, dose and route of exposure

B. abortus virulent strain 2308 was used in most of the trials [52.94% (27/51)] for the challenge (Figure 2 and 3). The second strain most used was *B. abortus* 544 (American Type Culture Collection – ATCC 23448), that was used in 18 trials [35.29% (18/51)], followed by the strain VRI3, used in 11.76% of the trials (6/51) (Table 1). The challenge dose was close to 10^7 CFU (9.4×10^6 to 5.2×10^7) in 43 trials [84.31% (43/51)], close to 10^8 CFU ($1.7 \times$ to 5×10^8) in 6 trials [11.76% (6/51)], and between 7.15 to 9×10^5 CFU in 2 trials [3.92% (2/51)] (Table 1, Figures 2 and 3). The route used for challenge was mostly intraconjunctival [88.23% (45/51)], followed by subcutaneous [7.84% (4/51)] and intramuscular [3.92% (2/51)] (Table 1 and Figure 2).

3.6- Post-vaccination serology and vaccine strain clearance

Twenty-nine trials [74.35% (29/39)] that used S19 performed post-vaccination serological tests. For antibody evaluation of S19 post-vaccination the most used serologic test was the Complement Fixation Test (CF) [72.41 % (21/29)], followed by the Rose Bengal Test (RBT) [58.62% (17/29)], the Standard Tube Agglutination Test (STAT) [58.62% (17/29)], the Indirect Hemolysis Test (IHLT) [20.68% (6/29)], Enzyme Linked Immunossorbent Assays (ELISAs) in 20.68% (6/29); the Rivanol Test [13.79% (4/29)]; whereas the 2-Mercaptoethanol Test (2-ME), the Radial Immunodifusion Test (RID), and the Particle Concentration Fluorescence

Immunoassay (PCFIA) were used in only one trial each [3.45% (1/29)]. For S19, the animals were seropositive from the second week after vaccination and all animals in all studies returned to negative results in serological tests from 3 to 58 weeks after vaccination, depending mainly on age at vaccination, the dose and the test(s) used (Table 2).

Of the trials that used RB51, 91.66% (11/12) performed post vaccination serologic tests. Most of them [72.72% (8/11)] used both STAT and RB51 dot blot tests to evaluate the non-seroconversion in conventional serological methods. Among the classic serological methods the most used was STAT [81.82% (9/11)], followed by RBT [27.27% (3/11)]; whereas CF, RID and 2-ME tests were used in one trial each [9.09 % (1/11)]. To evaluate RB51 seroconversion, the RB51 dot blot [81.82% (9/11)] and ELISA using RB51 antigen [18.18% (2/11)] were used.

The clearance of the vaccine strain was evaluated through multiple puncture of the superficial cervical lymph node by two trials that used S19 [5.12% (2/39)] (Cheville et al., 1993; Cheville et al., 1996) and by six that used RB51 [50.00% (6/12)] (Cheville et al., 1993; Cheville et al., 1996; Olsen et al., 1999; Olsen, 2000b). For S19, the vaccine clearance occurred from 6 to 12 weeks (average of 9 ± 3 weeks), whereas for RB51, the minimum clearance period was 6 weeks and the maximum over 14 weeks (average of 8.3 ± 3.66 weeks). The detailed data on post-vaccination serology and clearance are shown in Table 2.

3.7- Post-challenge serology

Regarding the post-challenge serology, in animals vaccinated with S19, this information could be extracted from only 9 trials [23.07% (9/39)] (Manthei et al., 1952; King & Frank, 1961; Confer et al., 1985; Cheville et al., 1993; Wyckoff et al., 2005) (Table 3). Of these, none reported the complete absence of the anti-*B. abortus* antibodies after challenge, and in all at least one animal reacted to the tests among those vaccinated. These trials used the following serological tests after challenge: RBT [55.55% (5/9)], STAT [44.44% (4/9)], Rivanol Test [44.44% (4/9)], CF [44.44% (4/9)], and Fluorescence Immunoassay (FI) [11.11% (1/9)]. Serology performed in vaccinated animals after challenge resulted in different outcomes, according to the time when it

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was performed, with the highest number of seropositive animals 2-4 weeks after challenge and the lowest 36 weeks after challenge (Wyckoff et al., 2005).

In animals vaccinated with RB51, 9 trials [75% (9/12)] (Cheville et al., 1993; Elzer et al., 1998; Olsen et al., 1999; Olsen, 2000a, 2000b; Poester et al., 2006) performed post-challenge serological tests, and none reported complete absence of anti-*B. abortus* antibodies in vaccinated animals after challenge. These trials used the following serological tests after challenge: STAT [88.89% (8/9)], RBT [22.22% (2/9)] and 2-ME [11.11% (1/9)]. The detailed data of the post-challenge serology are summarized in Table 3.

3.8- Assessment of protection against clinical signs

Among the trials that performed S19 vaccination, 28 [71.79% (28/39)] evaluated some brucellosis clinical sign after exposure to virulent *B. abortus*, including abortion *stricto sensu* [57.14% (16/28)], premature birth or weak calves [46.42% (13/28)] and stillbirths [17.85% (5/28)]. In 14 trials, the clinical signs were not detailed, being usually grouped by the selected study as "abortion" [50.00% (14/28)]. They are described in the Supplementary Table S6 in column "Total outcomes". From 2 studies [8.33% (2/24)] (5 trials) (Crawford et al., 1990; Cheville et al., 1996) that challenged pregnant animals, it was not possible to assess the data on protection against clinical signs (unavailable data or only showed in figures or in summary).

Among trials that performed RB51 vaccination, 10 out of 12 trials [83.33% (10/12)] assessed the occurrence of brucellosis clinical signs after challenge, 2 reported specifically the occurrence of premature or weak calves [20% (2/10)] and 1 abortion *stricto sensu*. Supplementary Table S6 shows the detailed data of clinical signs of bovine brucellosis (abortion *stricto sensu*, premature or weak calves and stillbirth) after challenge in vaccinated and control animals. Figure 4 summarize the results of the protection against abortion *lato sensu* according to vaccine strain and dose used.

The relationship between the stage of pregnancy at challenge and the gestational age of abortion *lato sensu* / delivery were assessed in 13 trials [13/39 (33.33%)] that used S19 vaccine. This data is shown in Supplementary Table S7.

342

343 *3.9- Assessment of protection against infection*

344 The protection conferred by brucellosis vaccines, assessed by the presence of bacteria in
345 the animal's tissues after challenge, was performed in all the selected studies. However, from two
346 studies (Woodard & Jasman, 1983; Tabynov et al., 2014a) the bacteriology data was not available
347 for the individual groups (vaccinated and control) (Figure 4). The *B. abortus* challenge strain was
348 isolated in 91.89% (34/37) of the trials that performed vaccination with S19 from at least one
349 animal among those vaccinated. In three trials [8.10% (3/37)], the authors stated that it was not
350 possible to isolate *B. abortus* from animal's tissues after vaccination with S19 (Sutherland et al.,
351 1981; Cheville et al., 1993; Montaña et al., 1998), although culture-positive animals were
352 observed among control group. Bacteriological tests after exposure to the challenge strain were
353 performed from different tissues, including maternal and fetal samples: 21 trials [53.84% (21/39)]
354 from fetus, 20 [51.28% (20/39)] from colostrum or milk; 14 [35.89% (14/39)] from vaginal
355 discharge or uterus; 10 [25.64% (10/39)] from lymph nodes; and 8 [20.51 % (8/39)] from fetal
356 membranes.

357 For the trials that used RB51, data on bacteriology analysis from animal's tissues after
358 challenge was obtained from all 12 trials assessed. From these, in 4 trials [33.33% (4/12)] *B.*
359 *abortus* (both challenge and vaccine strains) was not isolated from any tissues among vaccinated
360 animals only from control group (Cheville et al., 1993; Olsen, 2000b). Bacteriological tests after
361 challenge were performed from different tissues, including maternal and fetal samples: 8 [66.67%
362 (8/12 from fetus; 4 [33.33% (4/12)] from fetal membranes; 3 [25% (3/12)] from colostrum or
363 milk; 3 [25% (3/12)] from vaginal discharge or uterus; and 3 [25% (3/12)] from lymph nodes.

364 Supplementary Table S8 shows the detailed data on protection against infection according
365 to the vaccine strain (S19 and RB51) in the selected papers by trial, showing the bacteriologic
366 results after exposure to virulent *B. abortus* in maternal and fetal tissues. Figure 4 and
367 Supplementary Figure S1 summarize the abortion *lato sensu* and infection rates of vaccinated and
368 control groups according to vaccine strain and dose used.

369

370 *3.10- Meta-analysis*

371 For the meta-analysis regarding protection against reproductive clinical signs of brucellosis
372 (grouped as abortion *lato sensu*), a total of 12 papers (15 trials) were selected and divided into 4
373 groups according to vaccine strain and dose used: S19 10^8 CFU / dose (vaccinated with a dose
374 close to 10^8 CFU of S19); S19 10^9 CFU / dose (vaccinated with a dose close to 10^9 CFU of S19);
375 S19 10^{10} CFU / dose (vaccinated with a dose close to 10^{10} CFU of S19); and RB51 10^{10} CFU /
376 dose (vaccinated with a dose close to 10^{10} CFU of RB51). In all these meta-analysis groups,
377 animals were vaccinated subcutaneously, the challenge dose was close to or 1 x 10^7 CFU and all
378 animals were exposed to *B. abortus* between 5 and 7 months of pregnancy. For the meta-analysis
379 of protection against infection, a total of 17 papers (23 trials) were selected adding the group of
380 non-pregnant animals vaccinated with S19 10^{10} CFU / dose and challenged with a dose close to
381 or 1 x 10^7 CFU of virulent *B. abortus*. The RR and VE for abortion or *B. abortus* infection were
382 the summary measures calculated. The meta-analysis results are shown in the Figure 5 and Figure
383 6.

384 Overall, the protection against abortion *lato sensu* in vaccinated animals was similar (RR
385 = 0.41, 95% CI: 0.32 – 0.52; VE = 58.85%, 95% CI: 47.72 – 67.61) to protection against infection
386 (RR = 0.43, 95% CI: 0.35 – 0.52; VE = 57.32%, 95% CI: 47.51 – 65.30) compared with non-
387 vaccinated animals. The results of the meta-analysis showed that animals vaccinated with 10^{10}
388 CFU of S19 have 1.88 times less probability to abort (RR = 0.53, 95% CI: 0.40 – 0.71; VE =
389 47.13%, 95% CI: 29.35 – 60.44) compared with animals in control groups. Animals vaccinated
390 with 10^9 CFU of S19 exhibited 4 times less risk of abortion (RR = 0.25, 95% CI: 0.12 – 0.52; VE
391 = 75.09%, 95% CI: 48.08 – 88.05) after challenge, than non-vaccinated animals. The probability
392 of abortion after challenge was 2.5 (RR = 0.40, 95% CI: 0.21 – 0.75; VE = 60.00%, 95% CI:
393 25.02 – 78.66) times lower among vaccinated animals with 10^8 CFU of S19 compared with non-
394 vaccinated ones. For meta-analysis of trials that used the RB51, animals that received the vaccine
395 at the dose of 10^{10} CFU exhibited 3.22 (RR = 0.31, 95% CI: 0.16 – 0.61; VE = 69.25%, 95% CI:
396 39.48 – 84.38) times less probability of abortion after challenge, compared with non-vaccinated
397 animals.

Protection against infection was non-significant for the subgroups that used S19 at the doses of 10^8 (RR = 0.60, 95% CI: 0.27 – 1.35) and 10^{10} CFU (RR = 0.59, 95% CI: 0.34 – 1.05), including the non-pregnant animals vaccinated with S19 10^{10} CFU / dose and exposed to *B. abortus* (RR = 0.38, 95% CI: 0.13 – 1.10) compared with control groups after challenge. In contrast, S19 at 10^9 CFU (RR = 0.28, 95% CI: 0.14 – 0.55; VE = 72.03%, 95% CI: 57.70 – 81.50) and RB51 at 10^{10} CFU (RR = 0.43, 95% CI: 0.22 – 0.84; VE = 57.05%, 95% CI: 30.90 – 73.30) showed significant protection against infection after challenge compared with control groups.

A similar level of protection against abortion *lato sensu* (Cochrane's Q-statistic = 5.01, d.f. = 3, P = 0.1714) and infection (Cochrane's Q-statistic = 8.05, d.f. = 4, P = 0.0899) was observed considering all subgroups of vaccine strains and doses assessed. For those meta-analysis subgroups that showed significant RR, the 95% CI of VE against abortion *lato sensu* and infection for comparisons among different vaccine strains and doses are shown in Figure 7. Detailed results on the meta-analysis for comparisons among the subgroups for abortion *lato sensu* and infection are shown in the Supplementary Table S9.

4- Discussion

This systematic review and meta-analysis aimed to analysis the efficacy of S19 and RB51 vaccines in high quality studies, from 1952 to 2016, and recalculate the efficacy of these vaccines by means of a meta-analysis. The information provided by this study is essential to update the efficacy of the two most used vaccine strains against bovine brucellosis and to critically assess the controlled trials used to evaluate these vaccines, which will serve as an important learning experience for appraisal of future vaccines. Indeed, our results highlights the best vaccine dose for S19 (10^9 CFU) and RB51 (10^{10} CFU), as well as indicate an ideal doses, routes and ages (or stage of pregnancy) to perform vaccination and challenge of animals under controlled experimental settings.

The results of this study also allowed the recalculation of vaccines' efficacy at different doses for the target species, without the need to repeat such experiments, which are very expensive, time- and human resources-consuming, have ethical issues, and require large animal biosafety

level 3 facilities. By recalculating the efficacy of S19 and RB51 vaccines, our study provides very relevant information for brucellosis control and eradication programs worldwide that can drive adjustments in vaccination schemes and brucellosis control modelling. Since this meta-analysis was performed using studies in the target species, results are more directly applied to the development of new vaccines or to the optimization of existing vaccines for bovine brucellosis than those obtained from studies in mice (Carvalho et al., 2016). Albeit a systematic review has been published on the efficacy of brucellosis vaccines in natural hosts, in this study the efficacy was not recalculated according to the vaccine's target species, type of vaccine (attenuated, vector, DNA, etc.) and dose used (Carvalho et al., 2020). Moreover, from this study, it was also not possible to identify the trials used for meta-regression and the methodological quality employed was not optimal [inclusion / exclusion criteria and number of studies evaluated in each category (type of vaccine, host and dose) were unclear]. Therefore, a systematic review and meta-analysis on the main vaccines used in the control of bovine brucellosis worldwide was truly needed. The present study reduced most of the heterogeneity among experimental brucellosis vaccine evaluation by estimating vaccine effect into subgroups considering the vaccine and the dose used on each trial. Moreover, the heterogeneity was also taken into consideration by modelling data using fixed-effects (plural) and random-effects models as required. Hence, the design of the analyses of the present meta-analysis increases the confidence in the estimates of vaccine efficacy against bovine brucellosis. Our findings showed that the protection against abortion *lato sensu* was slightly superior (but non-significantly) to protection against infection for global meta-analysis data and for the two subgroups that yielded significant results in both outcomes (S19 10^9 CFU and RB51 10^{10} CFU). Importantly, despite S19 at the dose of 10^8 and 10^{10} CFU being non-protective against infection, it showed protection against abortion *lato sensu*, which is important in decreasing economic damage and the transmission chain by reducing environmental contamination (Knight-Jones et al., 2014).

A direct comparison among vaccine strains and doses, for those groups that showed a significant RR showed similar levels of protection against both, abortion *lato sensu* and infection, having S19 at 10^9 CFU and RB51 at 10^{10} CFU the lowest RR and, consequently, the highest VE,

besides smaller 95% IC (Figure 5, 6 and 7). Nevertheless, it is also critical to note that comparable efficacy was achieved with one dose of RB51 about ten times higher than the one S19 dose. Moreover, it is also worth to mention that albeit two RB51 doses have been assessed by the studies selected in this systematic review, the efficacy of RB51 at the dose 10^9 CFU (Olsen, 2000a, 2000b) was evaluated only by two studies, with a small number of animals (control = 21, vaccinated = 15) and trials (two trials). These numbers can be considered very small compared with the numbers of trials and animals included in the other meta-analysis subgroups, especially for S19 (Figures 5, 6 and 7). A meta-analysis with this limited number of trials and animals would yield results that could not be generalized, as they were obtained from a very narrow population (Borenstein et al., 2010). Moreover, these two RB51 trials exhibited results in opposite directions (Olsen et al. 2000a RR $\geq$ 1; Olsen et al. 2000b RR $\leq$ 1; for both abortion *lato sensu* and infection). According to the OIE, it is recommended to vaccinate cattle as calves (4-12 months of age) with RB51 at a 1-3.4 × 10^{10} dose, with revaccination from 12 months of age onwards with a similar dose to elicit a booster effect and increase immunity.

In contrast, the 10^{10} CFU dose for S19, albeit being the most robust group among the meta-analysis performed (greater number of trials [five for abortion and seven for infection] and animals [131 for abortion and 233 for infection]) (Figure 6), was the one with the lowest level of protection against abortion *lato sensu* (efficacy of 47%) (non-significant) and did not exhibit protection against infection among all evaluated subgroups. Importantly, it should be noted that the dose recommended by the OIE for vaccination of calves between 4 and 8 months by the subcutaneous route is 5-10 x 10^{10} CFU, whereas a reduced dose of 5 x 10^9 is only recommended for administration to cattle of any age as either one or two doses by the conjunctival route (OIE, 2016). These results could be explained considering that exposure to a high dose of the vaccine may lead to a downregulation of the immune system and, consequently, a lower protection rate (Siegrist, 2017). However, the absence of immunological assessments in most selected studies does not allow the drawing of more definitive conclusions in this regard, as well as it precludes the identification of correlates of protection.

Commented [JG14]: Since alittle bit speculative, I would suggest to delete (it does not add info to the manuscript, I think).

Commented [ED15R14]: I would keep these sentences although they seems speculative, as I think they point to what you should address from now on. I believe for brucellosis would be fantastic if we could have a correlate of protection to screen potential vaccine candidates.

Commented [JG16R14]: Ok, no problem.

Our findings raise an important concern about the use of S19, since many programs to control bovine brucellosis worldwide recommend the 10^{10} CFU dose of S19 for the immunization of their herds (Deqiu et al., 2002; Chand et al., 2014; Brasil, 2017). On the other hand, the results of this meta-analysis suggest that S19 vaccine should be used at a dose of 10^{9} CFU, which is 50-~~100~~ 80 times lower than the dose recommended by the OIE for subcutaneous administration. This raises an important question about the production of bovine brucellosis vaccines by countries, such as India, that have the challenge to produce enough vaccine to immunize a huge cattle herd (Rathod et al., 2016). Indeed, whether the S19 lower dose is implemented this would result in up to 50-~~100~~ 80 times greater vaccine production instantaneously.

Another very significant point of the present meta-analysis is that our results consider the outcomes observed in the control group and not only the outcomes among the vaccinated animals for calculating efficacy, which was originally done by only three (Crawford et al., 1990; Poester et al., 2006; Fiorentino et al., 2008) of the selected papers. Vaccine efficacy should be evaluated by calculating the RR or attributable fraction (VE), since these measures considers how much more likely it is that an animal will be protected, if vaccinated, compared with the non-vaccinated ones (Dohoo et al., 2009). The calculation of only simple proportions (as performed for most of the selected studies), that do not consider the outcomes in the control group to express the vaccines efficacy, overestimates the protection rates. The use of RR or VE to assess the protection rate of the brucellosis vaccines ~~dismiss~~ re-emphasize the need of having a minimal abortion rate among the non-vaccinated animals to consider a trial valid. In addition to the low analytical quality, a significant amount of studies used six or less animals per group (Cheville et al., 1993; Cheville et al., 1996; Montaña et al., 1998; Olsen, 2000b), making a robust statistical assessment difficult given the expected large individual variability (large CI) and the weight of each experimental unit. This situation reinforces the advantages of conducting a systematic review to have more robust and relevant data that allowed the drawing of more correct conclusions.

The most used vaccination route in the trials, for both S19 and RB51, was subcutaneous (85.71%), which can be explained due to its easy access in cows compared with oral and intraconjunctival routes. Regarding the vaccine strain, S19 was the most used among the trials

Commented [JG17]: If we refer to the OIE recommendation then, I think we should mention 50-80 times

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Commented [JG18]: I would disagree: a minimum of 60-80 % abortion rate should be observed in the control group, although the "ideal" situation would be 100% abortion in the control group as documented for the evaluation of Rev.1 in sheep and goats (Verger 1995).

Commented [ED19R18]: I totally agree with you. However, when we use RR to calculate the vaccine efficacy as we divide the incidence among the vaccinated by the incidence in control animals, lower is the rate of abortion in control group lower is the RR, then lower is the protection. So, if we use the right method to calculate the efficacy, the studies with low rate of abortion among control animals will be automatically penalized. So, what we don't need to concern about this rate although we can discuss it.

Commented [JG20R18]: I could not agree more with your comment. Actually, this means that one cannot dismiss abortion rate among the non-vaccinated animals (that would be a wrong message, I think), but the opposite: it is critically important.
So, I suggest changing "dismiss" by "re-emphasize"

(76.47%) mainly at a dose close to 10^{10} CFU, likewise for RB51 the dose close to 10^{10} CFU was mostly used. This large difference in the number of studies testing S19 and RB51 is probably due to the fact that S19 has been developed long before RB51 and that S19 is used as the reference vaccine in studies for testing new bovine brucellosis vaccine candidates, as recommended by OIE (OIE, 2016). The long-life span of S19 compared with RB51 may also explain the greater variability in the number of S19 doses tested. However, despite being an older vaccine, S19 is still very effective and widely used, besides being less expensive than RB51. The main context for the use of S19 against bovine brucellosis is in the disease control phase, in which massive vaccination is the main strategy to reduce the prevalence and incidence. At this stage, other control measures are often very expensive and difficult to implement, (Olsen & Stoffregen, 2005). In contrast, RB51 due its DIVA (Differentiating Infected from Vaccinated Animals) characteristic has replaced S19 use in some countries or regions with a low prevalence of bovine brucellosis (Dorneles et al., 2015a), as moving towards the eradication of bovine brucellosis requires a strict test-and-slaughter policy. In this phase, vaccination is usually forbidden and may be used only to contain outbreaks, preferably using RB51, as it does not interfere with the results of diagnostic tests. However, despite in some outbreaks situations, vaccination of the entire population is recommended (Dorneles et al., 2014), it is important to note that according to the OIE, both vaccines can be used in pregnant animals, however there is a risk of causing abortion (Dorneles et al., 2015a), although the rate of abortion by RB51 has been estimated as low as 0.5% (Sanz et al., 2010). To reduce the risk of abortion following S19 vaccination, a reduced dose from 3×10^8 to 5×10^9 CFU can be administered subcutaneously, but some animals can develop persistent antibody titers and may abort and excrete the vaccine strain in the milk (OIE, 2016).

In controlled clinical assays to evaluate the efficacy of vaccines against bovine brucellosis another critical aspect to be considered is the challenge with virulent *B. abortus*, including the strain, dose, route and animal status (pregnant or non-pregnant). The majority of the selected studies performed the challenge in animals between 4 and 7 months of pregnancy (64.70%), probably due to *B. abortus* tropism for the erythritol produced by the pregnant uterus, which favors the colonization by the microorganism (Smith et al., 1962), and also considering that the

Commented [JG21]: You mean shelf live?

Commented [ED22R21]: We want to say that S19 has been in use for longer than RB51, since 1950s

Commented [JG23R21]: OK

main clinical sign of brucellosis is abortion in the final third of pregnancy (Carvalho Neta et al., 2010). In fact, the challenge of non-pregnant animals has a very limited scope in brucellosis vaccine assessment, since it does not allow to investigate the vaccine's ability to avoid the reproductive clinical signs of the infection, important for causing economic losses and in the intra-herd spread of the disease. For non-pregnant animals, a separated subgroup meta-analysis was conducted, as these studies could not be grouped with others, because the physiology of the pregnant animal is very different from the non-pregnant ones (Wankhade et al., 2017).

Similarly to the stage when the challenge is performed, the dose used in the exposure is another important variable in these experiments, since the bacterial load influences the host-parasite interaction and thereby the vaccine efficacy (Nicoletti, 1990). Meta-analysis did not include experiments that used challenge doses of 10^8 CFU (Buddle, 1948; Olsen, 2000b; Tabynov et al., 2014a; Tabynov et al., 2014b; Tabynov et al., 2016), since previous studies have shown that the exposure to 10^7 CFU of virulent *B. abortus* (used by 83.67% of the studies) yield a degree of infection not different from those observed after natural infection (Fensterbank & Plommet, 1979); and small increases (less than a logarithm) in the challenge dose result in large increase in abortion in both, control and vaccinated groups (Manthei, 1959), which also precludes a significant analysis of vaccine efficacy.

Likewise, the challenge route is also an important aspect for experimental infections, since it should reproduce what happens in natural infection. For this reason, most of the studies (88.23%) carried out the inoculation of the virulent *B. abortus* by intraconjunctival route, considering that the microorganism is most frequently acquired by ingestion, followed by inhalation and conjunctival exposure (Corbel, 2006). On the contrary to the relevance of the dose, route and stage in which the challenge is carried out, the challenge strain does not seem to influence the evaluated outcomes, as previously demonstrated in mice (Miranda et al., 2015), being only author's discretion, as well as observed for the animal breed used.

Although the evaluation of the humoral immune response followed by vaccination has been evaluated by most trials, it should be noted that these data were poorly described and exceedingly difficult to interpret among those extracted from the selected papers. It is possible that the minor

importance given to these data occurred due to the already known secondary role of antibodies in the response against brucellosis (Dorneles et al., 2015b). For the S19 vaccinated animals, serological tests were used to make inferences about the clearance of antibodies induced by vaccination and to assess seroconversion post-challenge. For the first objective, studies evaluated the effect of age on vaccination or of S19 reduced dose and showed that the shortest time for the clearance of anti-S19 antibodies occurs in animals vaccinated between 6-12 months, and that vaccination with a reduced dose exhibited a shorter antibody clearance time compared with vaccination with the full dose (Cocks & Davies, 1973; Cheville et al., 1993; Cheville et al., 1996; Olsen & Stoffregen, 2005). Indeed, for S19, 60% (3/5) of the trials that had an antibody clearance time less than 10 weeks (Alton et al., 1980; Alton & Corner, 1981; Cheville et al., 1993; Fiorentino et al., 2008) used a vaccine dose close to 10^8 CFU (Alton et al., 1980; Alton & Corner, 1981) and 10^9 CFU (Alton et al., 1980; Cheville et al., 1993). On the other hand, one study (Fiorentino et al., 2008), although having used 10^{10} CFU of S19, demonstrated a clearance time under 8 weeks but, in this case, the animals were vaccinated at 6 months of age. In contrast to S19, the time required for the clearance of anti-RB51 antibodies has not been determined, as there is no cutoff point or validated tests for this proposal. RB51 clearance time (vaccine strain) was evaluated in 50% of the trials, by weekly lymph nodes puncture, being this analysis important to understand how long the vaccine stays in the host (residual virulence). This assessment is especially relevant in vaccination of older animals, considering that this strain can be shed in milk or even in vaginal secretion (Dorneles et al., 2015a). The age at vaccination was inversely proportional to the RB51 clearance time, since the trials that vaccinated animals at 18 months (Elzer et al., 1998; Olsen, 2000b) had a shorter clearance time than those that vaccinated animals at 7 months (Olsen et al., 1999) or 10 months (Cheville et al., 1993). Therefore, despite (Cheville et al., 1996) have stated that the age at vaccination does not interfere in the immune response following vaccination, the results of our systematic review lead us to infer that the clearance of the RB51vaccine strain is influenced by the age of the animal. For S19, there are not enough trials that performed this analysis to state whether animal age at vaccination influences the vaccine clearance time. These aspects might by clarified in future experimental studies.

Commented [JG24]: Perhaps worth to describe anti-rough LPS antibodies.

Commented [ED25R24]: See my other comment above

Commented [JG26R24]: Ok

Data on post-challenge serology was less available in the evaluated full-texts compared with post-vaccination data, the more complete results were obtained from King and Frank (1961), whom used the S19 vaccine at 5 x 10^{10} CFU dose and the lowest challenge dose (9 x 10^{5} CFU) among all trials, obtaining 28% seropositivity, and from Poester et al. (2006) that used RB51 vaccine at 1.5 x 10^{10} CFU dose and a challenge dose of 3 x 10^{7} CFU, obtaining 65% seropositivity. These differences in the seropositivity rate are certainly associated with the difference in challenge dose used between the studies, as well as with the timing post challenge when serology tests were performed or by the tests and cut-off points used. The first authors discusses that younger animals react less at the STAT after vaccination with S19 compared with animals at 9 months of age, leading to the inference that younger animals would have less problems with false-positive serological results when they reach the appropriate age for being tested, which is also stated by Poester et al. (2006)

The duration of the immunity conferred by bovine brucellosis vaccines was an interesting subject that could not be assessed by this systematic review. However, Manthei (1959) performed long longitudinal studies, demonstrating that protection conferred by a single dose of 1-1.2 x 10^{10} CFU S19 lasted longer than 10 years. Probably for this reason, most selected studies (82.75%) evaluated only the effect of a single dose of vaccine strains. In fact, as attenuated vaccines mimic natural infection, usually a single dose is necessary to confer long-lasting immunity (Dorneles et al., 2015a). The duration of immunity and the need for a boost vaccination after the subcutaneous administration of S19 at the dose of 10^{9} and RB51 at the dose of 10^{10} could not be assessed in this study.

In conclusion, our systematic review and meta-analysis suggest that the dose of 10^{9} CFU for S19 and 10^{10} CFU for RB51 (both administrated by subcutaneous route, at a single dose) are the most suitable for the prevention of abortion *lato sensu* and infection in cattle. In addition, in the selected controlled experiments the challenge was usually carried out intraconjunctivally by inoculation of 10^{7} CFU of *B. abortus* in the middle third of pregnancy and that the most used vaccination route was subcutaneous.

In light of the results of this study, the doses of bovine brucellosis vaccines recommended by the OIE should be revised. Indeed, in the case of S19, this would allow to commercialize 50-~~100~~ 80 times more doses for the same amount of CFU produced in countries were production capacity is a major constrain for implementing sound brucellosis control programs.

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Conflict of interests

The authors declare no competing interests.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

Data availability statement

The data that supports the findings of this study are available in the supplementary material of this article.

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Figure captions

Figure 1 – PRISMA flowchart used in the selection of the studies for this systematic review and meta-analysis.

Figure 2 – Experimental design of the 51 trials from 29 studies selected by this systematic review on the efficacy of bovine brucellosis vaccines.

Figure 3 – Alluvial diagram showing the main experimental design characteristics of the 51 trials from 29 studies selected by this systematic review on the efficacy of bovine brucellosis vaccines.

Figure 4 – Alluvial diagram showing abortion and infection rates of vaccinated and control groups according to vaccine strain and dose used, following the challenge with virulent *Brucella abortus* in the 51 trials from 29 studies selected by this systematic review.

Figure 5 – Meta-analysis data and forest plot graphics of protection against clinical signs of brucellosis (abortion *lato sensu*) after exposure to virulent *Brucella abortus* conferred by vaccination with S19 and RB51 at different doses. All the reproductive clinical signs reported in the articles, as stillbirth, born of weak calves, premature calves and abortion were considered as abortion.

Figure 6 – Meta-analysis data and forest plot graphics of protection against brucellosis infection after exposure to virulent *Brucella abortus* conferred by vaccination with S19 and RB51 at different doses. The data included the isolation of the challenge strain in any organ from the animals in the experiment, including fetal tissues.

Figure 7 – Comparison of vaccine efficacy (VE) among meta-analysis subgroups for protection against abortion *lato sensu* (A) and infection (B) conferred by vaccination with S19 and RB51 at different doses after exposure to virulent *Brucella abortus*, for those subgroups that showed significant risk ratio.

Supplementary Figure S1 – Alluvial diagram showing abortion and infection rates of vaccinated and control groups according to strain and dose used, in both vaccination and challenge, in the 51 trials from 29 studies selected by this systematic review.