MARINA MARTINS DE OLIVEIRA

EFFICACY OF *Brucella abortus* S19 AND RB51 VACCINE STRAINS:

A SYSTEMATIC REVIEW AND META-ANALYSIS

Lavras – MG

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Thesis submitted to the Universidade Federal de Lavras, as a partial requirement for obtaining the title of PhD in Veterinary Sciences.

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EFICÁCIA DAS CEPAS VACINAIS B19 E RB51 CONTRA *Brucella abortus*: UMA REVISÃO SISTEMÁTICA E METANÁLISE

Thesis submitted to the Universidade Federal de Lavras, as a partial requirement for obtaining the title of PhD in Veterinary Sciences.

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LIST OF FIGURES

subgroups that showed significant risk ratio. All reproductive clinical signs reported in the articles, as stillbirth, born of weak calves, premature calves and abortion were considered as abortion *lato sensu.* The data included the isolation of the challenge strain in any organ from the animals in the experiment, including fetal tissues. k – number of trials.

Supplementary Figure S1 – Alluvial diagram showing infection and abortion 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.

LIST OF TABLES

Supplementary Table S8 – Detailed data on protection against infection after challenge with virulent *Brucella abortus* according to trials that performed this analysis among those selected by this systematic review.

Supplementary Table S9 – Detailed results on the meta-analysis for comparisons among the subgroups for abortion and infection.

RESUMO

A brucelose bovina é uma zoonose que atinge o mundo todo, mais frequentemente países em desenvolvimento, e a vacinação dos bovinos e bubalinos com as vacinas B19 ou RB51 é uma das principais formas de prevenção da doença. Este estudo teve como objetivo revisar a literatura que estimou a eficácia de ambas as vacinas mais utilizadas contra a brucelose bovina, a fim de reunir as principais informações de estudos experimentais, como dose, via de administração, dose desafio, entre outras, além de recalcular o nível de proteção das duas principais vacinas para bovinos. A amostra vacinal mais utilizada foi a B19, na dose de 10^{10} unidades formadoras de colônias (UFC), seguida da amostra vacinal RB51 a 10^{10} UFC. A amostra de desafio mais utilizada foi *B. abortus* 2308, na dose de 10^{7} UFC por via intraconjuntival. Foi realizada metanálise Ppara o recálculo da eficácia das vacinas, verificando-se que a vacina B19 na dose de 10^{9} UFC apresentou maior proteção contra infecção e aborto que as demais doses vacinais, enquanto a vacina RB51 na dose de 10^{10} UFC exibiu maior proteção contra ambos os sinais clínicos e infecção do que a outra dose da mesma vacina. Foi possível concluir que a vacina B19 na dose de 10^{9} UFC e a vacina RB51 na dose de 10^{10} UFC administradas por via subcutânea foram as mais eficazes para prevenir aborto e infecção contra *Brucella abortus* 2308 utilizada no desafio experimental na dose de 10^{7} UFC por via intraconjuntival.

Palavras-chave: brucelose bovina, vacinação, aborto, infecção, proteção.

ABSTRACT

Bovine brucellosis is a zoonosis that affects the whole world, most often in developing countries, and vaccination with the S19 or RB51 vaccines is the main way to prevent the disease. This study aimed to review the literature that estimated the effectiveness of both of the most used vaccines against bovine brucellosis in order to gather the main information from experimental studies, such as dose, route of administration, challenge dose, among others, in addition to recalculating the effectiveness of the two main vaccines for cattle. The most used vaccine strain was S19, at a dose of 10^{10} colony forming units (CFU), followed by the RB51 vaccine strain at 10^{10} CFU. The most used challenge strain was *Brucella abortus* 2308, at a dose of 10^{7} CFU by intraconjunctival route. For the recalculation of vaccine efficacy, a meta-analysis was performed, in which the main results were that the S19 vaccine at a dose of 10^{9} CFU presented greater protection against infection and abortion than the other vaccine doses, while the RB51 vaccine at a dose of 10^{10} UFC exhibited greater protection against both clinical signs and infection than the other dose of the same vaccine. It was possible to conclude that the S19 vaccine at a dose of 10^{9} CFU and the RB51 vaccine at a dose of 10^{10} CFU administered subcutaneously were the most effective to prevent abortion and infection against the experimental challenge with *Brucella abortus* 2308 at a dose of 10^{7} CFU by intraconjunctival route.

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

SUMMARY

1 INTRODUCTION

Bovine brucellosis is an infectious disease caused by *Brucella abortus* and has been reported as the most prevalent zoonosis worldwide (Cross et al., 2019). It is highly contagious among animals, since a low infectious load is necessary to cause the transmission by aerossol (Bossi et al., 2004) and the abortion of an infected animal, that is an ordinary clinical sign (Corbel, 2006), can discharge a considerable infectious load [around 10^{14} colony forming units (CFU/g of fetus tissue)] of *Brucella* (Alexander, Schnurrenberger and Brown, 1981; Corner, 1983). Although brucellosis has been eradicated from some countries in Europe and North America (Godfroid and Kasboher, 2002), the disease is still prevalent in developing countries, including those in South America and Africa (Poester, Samartino and Santos, 2013).

The vaccination is the most important measure to control the disease in bovines and the current vaccines most used are S19 and RB51 (Dorneles, Sriranganathan and Lage, 2015). The S19 was developed in 1941 from a smooth naturally attenuated *B. abortus* strain, Although this vaccine caninduce antibody response that can cause a misinterpretation on diagnosis tests for the disease (Manthei, 1959), if vaccination is performed in animals older than 8 months. The RB51 vaccine was developed in 1982 and is a rough rifampicin resistant strain that does not express the O-chain of the lipopolysaccharide (LPS) on its outer membrane, thus, it does not induce the detectable antibodies (Schurig et al., 1991). For this reason, the S19 vaccine is recomended for animals from 3 to 8 months of age, while RB51 vaccine can be used in animals at any age (Dorneles, Sriranganathan and Lage, 2015). However, it is important to consider that in cattle herds animals are pregnant and in lactation most of time, and both vaccine strains are not recommended for pregnant animals and may be shed in milk when vaccination is performed in early lactation (Miranda et al., 2016). In this scenario, brucellosis vaccination is usually performed in female calves.

Meantime, there is no consensus about neither the efficacy of the vaccines nor the ideal age for vaccination. Manthei (1959) stated that the S19 protect around 65-75% of the animals, while RB51 has around 95% of efficacy, depending on the age at vaccination (Olsen and Stoffregen, 2005). Since 1948 (Buddle, 1948), there are a considerable literature that performed very important experiments on brucellosis vaccines efficacy that can be very useful to conduct a meta-analysis to recalculate their efficacy to form a consensus on some aspects of bovine brucellosis vaccination. In this context, some studies

have shown an average of vaccination protection of about 65-75% (Manthei et al., 1952; Nicoletti, 1990; Olsen, 2000; Olsen & Stoffregen, 2005; Poester et al., 2006) of either S19 or RB51, but the calculation of vaccine efficacy in most of published studies is inappropriate, as it does not take into account results in control groups. Moreover, there is still some discussions on the ideal vaccine dose and route, the challenge dose, the stage of pregnancy at challenge (in experimental studies), among other factors that need to be assessed to design optimized brucellosis vaccine assessment assays, which can be used for testing new vaccine candidates. Still, researches involving cattle are generally expensive, time and human resources consuming, besides ethically complicated, rendering difficult new experiments on this subject. In this context, a systematic review can help to assess the importance of different variables for both vaccines, while a meta-analysis can be used to recalculate vaccine efficacy, using a more robust number of animals and minimizing calculation errors by disregarding control groups.

2 THEORETICAL REFERENCE

2.1 Importance to public, animal and financial health of bovine brucellosis

Brucellosis is an infectious zoonotic disease of worldwide importance caused by the microorganisms of *Brucella* genus, which are small coccobacilli (0.4-3.0 μm), pleomorphic, facultative intracellular, Gram-negative, no encapsulated, non-motile, and have the ability to invade, survive for long periods and multiply in host cells (Poester et al., 2013). At the environment remain viable for long periods, in humid environments and with organic matter, without direct sunlight and at neutral pH; for example, remain viable for up to eight months (Aparicio, 2013; Corbel, 2006; Lage et al., 2008; MAPA, 2006; Olsen & Tatum, 2010). They are no longer resistant to heat and direct sunlight, being destroyed by pasteurization (MAPA, 2006). *Brucella* spp. do not have specific hosts, but have a predilection species: cattle are mainly affected by *B. abortus*, goats by *B. melitensis*, sheep by *B. ovis*, swine by *B. suis* and canids by *B. vulpis* (Corbel, 1997; Paulin and Ferreira Neto, 2003). The disease has a subacute or chronic course, which infects a wide variety of wild and domestic animals, as well as human beings (Corbel, 2006).

Brazil occupies a prominent position in the world in the production and export of beef, considering that only in the third quarter of 2020, 7.69 million heads were under some type of sanitary inspection (IBGE, 2020). In 2021, there were 187.55 million heads of cattle in the country, and regarding the dairy activity, despite the impacts caused by the COVID-19 pandemic, in the third quarter of 2020 the acquisition of raw milk by establishments that are under some type of sanitary inspection grew 163.81 million liters of milk, representing an increase of 2.6% when compared to 2019. According to ABIEC (2021), from January to September 2021 there was a 10.79% growth in Brazilian agribusiness Gross Domestic Product (GDP) compared to the same period in 2020. Thus, it is necessary that the country meets the sanitary requirements not only of the domestic market as well as the foreign market, which requires, in addition to foot and mouth disease-free herds, herds and animal products that are vigilant in relation to bovine tuberculosis and brucellosis (Miranda et al., 2008).

In Brazil, *B. abortus* is endemic and disseminated in all territory (Poester et al., 2002), with a prevalence of positive herds ranging from 0.91%, in Santa Catarina, to 30.6%, in Mato Grosso do Sul (Ferreira Neto et al., 2016). Since 2001, Brazil started the National Program for the Control and Eradication of Brucellosis and Tuberculosis (Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose - PNCEBT),

which aims to reduce the prevalence and incidence of brucellosis and tuberculosis in the country. In cattle, the infection is mainly caused by *Brucella abortus* and the economic losses associated with bovine brucellosis are mainly related to reproductive problems, such as abortion, stillbirth, birth of weak calves, infertility, placental retention and culling of positive animals (Poester, Samartino & Santos, 2013).

The main clinical signs of brucellosis in female cattle is reproductive failure, as abortion, birth of weak calves, stillbirths, retention of fetal membranes, endometritis and reduction in milk production (Kiros, et al., 2016), while in male animals, the manifestations are orchitis and epididymitis, mostly (Corbel, 1997). When the animal is born at term, it can dead very soon after birth, with fibrinous pleuritis and interstitial pneumonia, impairments that may also be present in an aborted fetus (Kiros et al., 2016). The animals can be infected early in life, but no symptoms are visible until the animals reach reproductive age (Abdisa, 2018; Kiros et al., 2016). Besides that, the disease represents a risk not only for animals but also for human beings, in which the disease can be transmitted by direct or indirect contact with infected animals or their products, or through the ingestion of meat and unpasteurized milk (Pappas et al., 2005). In this context, in addition to economically harming livestock activity, it also affects public health.

In addition to that and considering the chronic nature of brucellosis in cattle, it is necessary to highlight the influence of the disease in the productive indices of the cattle production. A study conducted in Spain indicate a decrease in meat production from 5 to 15% and milk production from 10 to 25%; other than that, increase in interval between deliveries from 11.5 to 20 months;15% reduction in calf production; and increase of replacement rate of females by 15% (Bernués, Manrique and Maza, 1997). In the analysis of the cost of brucellosis to the producer, it can be counted expenses with medicines, veterinarians, depreciation of the value of animals from infected herds (and even of the whole herd). These economic losses in Brazil were estimated at R$420.12 in females over 24 months of age, infected in dairy herds and, in 2013, it was estimated that the total loss had been R$892 million due to the disease (Santos et al., 2013). Furthermore, the same authors report that for every 1% reduction in the prevalence of the disease, it is possible to save about R$155 million in the cost that bovine brucellosis has for Brazil.

It is considered an occupational disease, since there is a greater risk of infection for groups that deal directly with animals, such as farmers, handlers, slaughterhouse workers, veterinarians and laboratory workers (Corbel et al., 2006; Lage et al., 2008; Pereira et al., 2020; Santos et al., 2007). In this context, there is still an aggravating factor

for the disease in human beings, considering that there is no effective vaccine against brucellosis for this species.

2.2 The main means of controlling the disease: vaccination

The main measure for the control of brucellosis in the country was the implementation of compulsory vaccination of calves aged 3-8 months with S19 (Brasil, 2001). The measures to control bovine brucellosis are of paramount importance due to the possible financial and health problems caused by the disease. Summarily, it is possible to establish a good control with two basic measures, which are hygiene and vaccination. (Lage et al., 2005). Vaccination is important in the disease control, especially in developing countries, where bovine brucellosis occurs more frequently (Olsen and Stoffregen, 2005), while hygiene has the important role of preventing susceptible animals from being exposed to the microorganism and it includes all the processes: diagnosis through the isolation of the agent; restriction of animal movement by exchange or sale; and slaughter of positive animals (Lage et al., 2005).

About the disease diagnosis, according to PNCEBT, the official tests for brucellosis in cattle and buffalo is the milk ring test (MRT) for screening in dairy herds approach, and the Rose Bengal Test (RBT) for individual approaches; and 2-mercaptoethanol (2-ME), Standard Agglutination Tube (SAT), Complement Fixation (CF) and the Fluorescence Polarization Test (FPA), being the last one possible to use as unic test, to confirm the diagnosis (Brasil, 2001). The serodiagnosis of bovine brucellosis is still a challenge and vaccination is considered one of the most effective measures to reduce the prevalence of bovine brucellosis, being used in many disease control and eradication programs (Dorneles, Sriranganathan & Lage, 2015).

In Brazil, the vaccines that have the best results in preventing the disease are formulated with live attenuated strains of *B. abortus* (Dorneles, Oliveira & Lage, 2017), with the S19 and RB51 strains being widely used to control the disease in the world, demonstrating effectiveness in preventing infections. abortion and infection, as well as offering lasting protection (Poester et al., 2006). There are two other vaccine strains, SR82 and 45/20, but these are currently in disuse (Dorneles, Oliveira & Lage, 2017).

The S19 vaccine strain is an attenuated smooth organism that has been used for the prevention of bovine brucellosis for over seven decades. As an advantage, this vaccine has low pathogenicity, stability, in addition to high antigenicity and immunogenicity

(Corbel, 2006; Brasil, 2004a). However, this vaccine induces a serological response that cannot be differentiated from antibody responses caused by natural infection by wild-type strains (Dorneles, Sriranganathan & Lage, 2015). Among the properties of this strain is the presence of the O side chain, which is an immunodominant antigen present in lipopolysaccharide (LPS), to which most detectable antibodies in diagnostic tests are directed. In this way, vaccination stimulates the production of antibodies no differentiable from those produced in the natural infection (Corbel, 2006). Therefore, with the intention of drastically reducing this problem, female animals should be vaccinated with S19 at ages between 3 and 8 months, because upon reaching 24 months (the age defined for the official tests to be carried out) the level of vaccine antibodies tends to be baseline, not interfering with routine exams (Dorneles et al., 2015a).

The RB51 vaccine was developed in 1982 and comes from a rough, live attenuated rifampicin-resistant strain of *B. abortus* biovar 1 that does not express O chain on its surfaceLPS , or contains an insufficient amount to induce the formation of specific antibodies (Dorneles et al., 2015a; Olsen & Stoffregen, 2005). Thus, vaccination with RB51 does not induce antibodies against LPS detectable by routinely used serological tests, which allows both vaccination and the test and slaughter policy to be carried out at any age. Strain RB51 is a naturally occurring crude mutant derived from the smooth and virulent strain of *B. abortus* 2308 by multiple passages in media with subinhibitory concentrations of rifampicin and penicillin (Schurig et al., 1991). The immune response induced by this vaccine is based on a strong Th1 response and increased production of $TCD4^+$ and $TCD8^+$ cells, with production of IFNγ and IL-4 (Dorneles, Sriranganathan & Lage, 2015).

Regarding human beings, both vaccines are pathogenic and can cause the disease and, consequently, fever, nocturne sweating, weakness, wight loss and pain (in head, joint, muscles, abdomen, and back) in cases of vaccination accidents (Dorneles et al., 2015a). For RB51, because of it resistant to rifampicin, the antibiotic of choice for the treatment of human brucellosis, this vaccine strain is an even more dangerous strain in vaccine accidents (Dorneles, Sriranganathan & Lage, 2015). To avoid them, personal protective equipment such as gloves, goggles and N95 masks must be used by the veterinarian during the vaccination process (Dorneles et al., 2015a).

Due to the importance that brucellosis has around the world, much effort has been made to control or even eradicate the disease in cattle, which is considered a great challenge. Nowadays, the S19 vaccine is used at a dose of 5-8 x 10^{10} colony forming units

(CFU), according to World Organization for Animal Health (OIE) (OIE, 2016), and RB51 at a dose of 1-3.4 x 10^{10} CFU, the second one at the age of 4 to 12 months. Moreover, despite being less practicality and ease, 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 (OIE, 2016; Nicoletti, 1990). Despite the situation with anamnestic antibodies previously mentioned, in an outbreak situation, vaccination can be recommended in adult animals (Dorneles et al., 2014), considering that the abortion rate of the RB51 vaccine has been estimated at only 0.5% when applied to pregnant females (Sanz et al., 2010). To reduce the risk of miscarriages, a reduced dose of S19 ($3x10^8$ to $5x10^9$ CFU) can be used subcutaneously, but even with this lower dosage, the problems with vaccine antibodies still persist (OIE, 2016). In summary, despite the efficacy of both vaccine strains and long-term protection, some disadvantages persist, such as the possibility of identifying false positive animals in serological tests, the risk of infection in humans due to the pathogenicity of the strains and the abortifacient potential in cows (Dorneles, Sriranganathan and Lage, 2015).

As mentioned earlier, vaccination reduces the prevalence of the disease especially in countries where control is desired. The vaccination objective is to reduce the number of animals susceptible to infection, reaching the level of eradication when combined with vaccine coverage and efficacy. Vaccines were evaluated for their efficacy in three phases: the first in laboratory animals; the second in natural but experimentally challenged hosts; and the last in natural hosts with environmental (natural) challenge (Nicoletti, 1990). In experimental studies, a virulent *B. abortus* is inoculated, mostly by intraconjuntival route, to generate the disease in order to assess clinical signs and infection to verify the rate of vaccine protection. In this context, the challenge is mostly performed with *B. abortus* strains 2308 or 544, both being similar with respect to their virulence (Miranda et al., 2015). The chosen challenge strain must be inoculated in pregnant animals between 4 and 7 months of pregnancy, considering that the physiology of a pregnant and a non-pregnant animal are very different (Wankhade et al., 2017), especially in relation to brucellosis: there is greater tropism of *Brucella* by the gravid uterus due to the erythritol produced by it, favoring the colonization of microorganisms (Smith et al., 1962) and also considering that it is in the final third of pregnancy that abortion takes place (Carvalho Neta et al., 2010). The vaccine efficacy (or protection) is estimated by the vaccine's ability to prevent infection and abortion (Olsen and Stoffregen, 2005).

Meantime, there is no consensus about neither the efficacy of the vaccines nor the ideal age for vaccination. Manthei (1959) found out that S19 efficacy was 65-75% (Manthei, 1959) and Olsen and Stoffregen (2005) that animals vaccinated with RB51 were protected with 95% of efficacy, depending on the age at vaccination. Assuming that brucellosis prevention is important for both animal and public health, both persistence of vaccine antibodies and vaccine efficacy need to be clarified, and in this context, a systematic review helps 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.

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

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Short running tittle: Efficacy of *Brucella abortus* vaccines

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Abstract

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 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, 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). 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). 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 infection and abortion 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, 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 5-8 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. 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.

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 Portuguese were excluded. Full inclusion and exclusion criteria are shown in the Supplementary Table S3.

2.3- Type of studies

Original experimental studies were included. Papers as cohort, case-control, cross sectional, case series, case reports and reviews were excluded.

2.4- Data extraction

Data were extracted from papers by one of the reviewers (MMO) and then checked for accuracy by another reviewer (EMSD). Disagreements regarding data extraction among reviewers were solved by consensus. Extracted data included: first author, year of the publication, geographic location, breed of animals, number of animals used, number of animals per group, animals age at vaccination, animals age at pregnancy, vaccine strain(s), vaccine dose, vaccine route, number of vaccinations, interval between vaccination(s) and challenge, pregnancy stage at challenge, challenge strain, challenge dose, challenge route, data on protection against clinical signs (abortion, stillbirth and

weak calves), data on protection against infection (maternal and fetal bacteriology), vaccine clearance and serologic response post vaccination and post challenge. Experimental studies without control groups or that did not report pregnancy stage or age of animals at challenge, vaccine dose, strain, and route, challenge dose, strain, and route, and either clinical protection (reproductive signs) or infection protection were excluded.

2.5- Meta-analysis

The trials were grouped for the meta-analysis based on their similarity regarding vaccine strain and dose, and stage of pregnancy at challenge. Only data from single vaccination were included in the meta-analysis. Moreover, for all meta-analysis groups, vaccination was performed by subcutaneous route, the challenge dose was close to or 1 x 10^7 CFU and all animals were exposed to virulent *B. abortus* between 4 and 7 months of pregnancy (Manthei, 1959; Nicoletti, 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 ratio (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 (R Core Team, 2021), using the packages meta (Balduzzi et al., 2019) and dmetar (Harrer et al., 2019), and the forest plots were produced using the packages meta and metafor (Viechtbauer, 2010).

3- Results

3.1- Selected studies

The literature review included papers published between 1948 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.

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

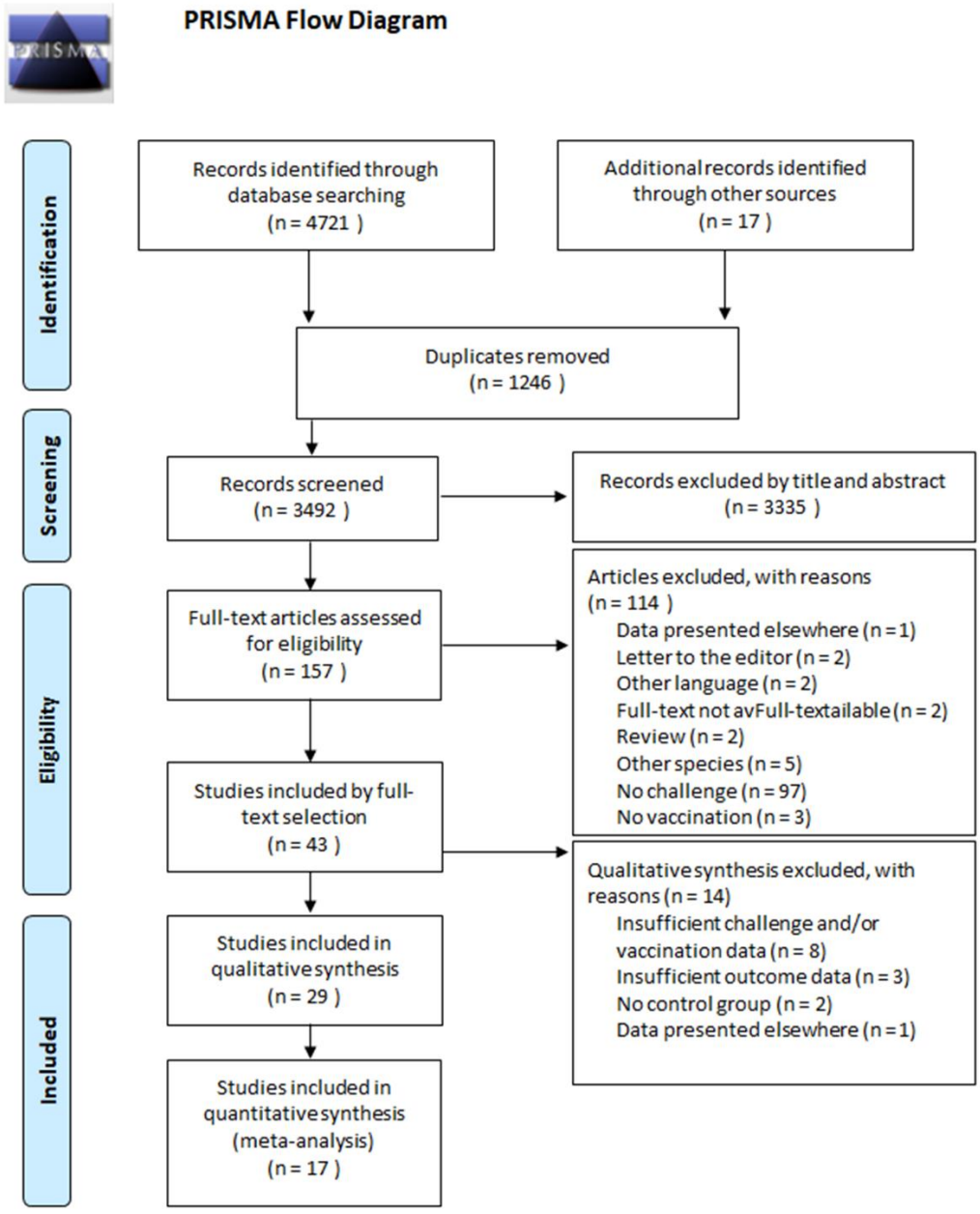


Table 1 – Vaccination and challenge data from trials selected in this systematic review on efficacy of bovine brucellosis vaccines (S19 and RB51).

First author, year	N Vac[a]	N C[b]	Vaccination				Challenge			
			Strain	Age	Dose	Route	Strain	Stage/Age	Dose	Route
Alton, 1980[c]	9	9	S19	3-4 preg[d]	2.8×10^8	SC[g]	VRI3	6-7 preg	2×10^7	IC[h]
Alton, 1980[c]	9	9	S19	3-4 preg	5.6×10^9	SC	VRI3	6-7 preg	2×10^7	IC
Alton, 1981	10	10	S19	14-23 m[e]	2.25×10^8	SC	VRI3	4.5-6.5 preg	1.3×10^7	IC
Alton, 1983	10	5	S19	15 m	3×10^8	SC	VRI3	4.8-6.8 preg	1.3×10^7	IC
Buddle, 1948	48	44	S19	6 m	1.85×10^{10}	SC	544	3.5-5 preg	1.7×10^8	IC
Buddle, 1948	42	44	S19	6 m	1.85×10^{10}	ICD[i]	544	3.5-5 preg	1.7×10^8	IC
Cheville, 1993	6	5	S19	10 m	$3\text{-}10 \times 10^9$	SC	2308	5 preg	1×10^7	IC
Cheville, 1993	4	5	RB51	10 m	$1\text{-}1.4 \times 10^{10}$	SC	2308	5 preg	1×10^7	IC
Cheville, 1996	16	15	S19	3-10 m	$1.31\text{-}1.71 \times 10^{10}$	SC	2308	5-6 preg	1×10^7	IC
Cheville, 1996	25	15	RB51	3-10 m	$1\text{-}1.4 \times 10^{10}$	SC	2308	5-6 preg	1×10^7	IC
Cocks, 1973[f]	11	11	S19	4-5 m	1.07×10^{11}	SC	544	13-14 m	2.15×10^7	IC
Confer, 1985	11	9	S19	10-12 m	1×10^9	SC	2308	4-5 preg	9.4×10^6	IC
Confer, 1985	10	9	S19	10-12 m	1×10^{10}	SC	2308	4-5 preg	9.4×10^6	IC
Confer, 1985	10	9	S19	10-12 m	1×10^9	SC	2308	4-5 preg	5.2×10^7	IC
Confer, 1985	8	9	S19	10-12 m	1×10^{10}	SC	2308	4-5 preg	5.2×10^7	IC
Crawford, 1990	40	69	S19	12 m	1×10^8	SC	2308	1.5-7.5 preg	1×10^7	IC
Crawford, 1990	39	69	S19	12 m	1×10^9	SC	2308	1.5-7.5 preg	1×10^7	IC
Crawford, 1990	39	69	S19	12 m	1×10^{10}	SC	2308	1.5-7.5 preg	1×10^7	IC
Davies, 1980[f]	10	10	S19	3-6 m	9×10^7	SC	544	13-16 m	1×10^7	IC
Davies, 1980[f]	9	10	S19	3-6 m	4.5×10^9	SC	544	13-16 m	1×10^7	IC
Davies, 1980[f]	10	10	S19	3-6 m	9×10^{10}	SC	544	13-16 m	1×10^7	IC
Elzer, 1998	10	10	RB51	18 m	3×10^{10}	Oral	2308	6 preg	2×10^7	IC
Fensterbank, 1979[m]	22	6	S19	6.5-9 m / 12.5-15 m	1.15×10^{11} / 5.7×10^9	SC / IC	544	6 preg	1.48×10^7	IC
Fensterbank, 1979[m]	22	6	S19	6.5-9 m / 12.5-15 m	6.1×10^9 / 5.7×10^9	IC / IC	544	6 preg	1.48×10^7	IC

Fiorentino, 2008	14	12	S19	6 m	2×10^{10}	SC	2308	5-6 preg	3×10^{7}	IC
King, 1961	14	2	S19	3-9 m	5×10^{10}	SC	2308	4-5 preg	$7.15\text{-}9 \times 10^{5}$	IC
Manthei, 1952	18	31	S19	12-15 m	$1.1\text{-}1.2 \times 10^{10}$	SC	2308	3-6 preg	$1.6\text{-}2.6 \times 10^{7}$	IC
Manthei, 1952	21	31	S19	12-15 m	$1.1\text{-}1.2 \times 10^{10}$	ID[j]	2308	3-6 preg	$1.6\text{-}2.6 \times 10^{7}$	IC
Montaña, 1998[f]	2	3	S19	19 m	2×10^{10}	SC	2308	21 m	1×10^{7}	IM[k]
Montaña, 1998[f]	3	3	RB51	19 m	2×10^{10}	SC	2308	21 m	1×10^{7}	IM
Olsen, 1999	12	6	RB51	7 m	$1.6\text{-}3.2 \times 10^{10}$	SC	2308	6 preg	1×10^{7}	IC
Olsen, 2000a	6	15	RB51	3 m	1.04×10^{9}	SC	2308	6 preg	1×10^{7}	IC
Olsen, 2000a	26	15	RB51	3-6 m	$1.09\text{-}1.22 \times 10^{10}$	SC	2308	6 preg	1×10^{7}	IC
Olsen, 2000b	7	6	RB51	18 m	1×10^{9}	SC	2308	6 preg	1×10^{7}	IC
Olsen, 2000b	4	6	RB51	18 m	3×10^{9}	SC	2308	6 preg	1×10^{7}	IC
Olsen, 2000b[m]	4	6	RB51	18 m / 19.5 m	1×10^{9} / 1×10^{9}	SC / SC	2308	6 preg	1×10^{7}	IC
Plackett, 1980	18	9	S19	0.8-5 m	9×10^{10}	SC	VRI3	5-6 preg	2×10^{7}	IC
Plackett, 1980[m]	10	9	S19	3-5 w[l] / 12 m	9×10^{10} / 4.5×10^{9}	SC / IC	VRI3	5-6 preg	2×10^{7}	IC
Plommet, 1976	12	7	S19	7-12 m	9×10^{10}	SC	544	4.5-6.5 preg	1.64×10^{7}	IC
Plommet, 1976[m]	12	7	S19	7-12 m / 13-20 m	9×10^{10} / 5×10^{9}	SC / IC	544	4.5-6.5 preg	1.64×10^{7}	IC
Plommet, 1976[m]	19	7	S19	7-12 m / 13-20 m	5×10^{9} / 5×10^{9}	IC / IC	544	4.5-6.5 preg	1.64×10^{7}	IC
Poester, 2006[n]	20	13	RB51	24 m	1.5×10^{10}	SC	2308	6-7 preg	3×10^{7}	IC
Renoux, 1964[f]	20	20	S19	7-9 m	6×10^{10}	SC	544	10-12 m	1.5×10^{7}	IC
Sutherland, 1981	7	8	S19	3-6 m	4×10^{10}	SC	544	3 preg	1×10^{7}	IC
Sutherland, 1981	11	8	S19	14-16 m	4×10^{10}	SC	544	3 preg	1×10^{7}	IC
Tabynov, 2014a	5	5	S19	12-18 m	8×10^{10}	SC	544	14-22 m	5×10^{8}	SC
Tabynov, 2014b	9	10	S19	3-4 preg	8×10^{10}	SC	544	5-6 preg	5×10^{8}	SC
Tabynov, 2014b	10	10	RB51	3-4 preg	3.4×10^{10}	SC	544	5-6 preg	5×10^{8}	SC
Tabynov, 2016	8	7	S19	3-4 preg	8×10^{10}	SC	544	5-6 preg	5×10^{8}	SC
Woodard, 1983	12	18	S19	12 m	5.9×10^{7}	SC	2308	3.5-5 preg	2.55×10^{7}	IC
Wyckoff, 2005[m]	7	9	S19	9-10 m / 11-13 m	1×10^{7} / 1×10^{7}	SC / SC	2308	4-6 preg	9.1×10^{5}	IC

[a]N Vac: number of vaccinated animals; [b]N C: number of control animals; [c]These trials used animals that were not in their first pregnancy; [d]preg: pregnancy; [e]m: months; [f]These trials challenge non-pregnant animals; [g]SC: subcutaneous; [h]IC: intraconjunctival; [i]ICD: intracaudal; [j]ID: intradermal, [k]IM: intramuscular; [l]w: weeks; [m]These trials performed a booster vaccination; [n]In this trial, 8 animals were vaccinated during early pregnancy. The vaccine and challenge doses are in CFU (colony forming unit).

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 at 1 x 10^9 CFU, in both doses, 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).

Figure 2 – Experimental design of the 51 trials from 29 studies selected by this systematic review on the efficacy of bovine brucellosis vaccines. Revaccination, for the trials that performed it, is shown in box.

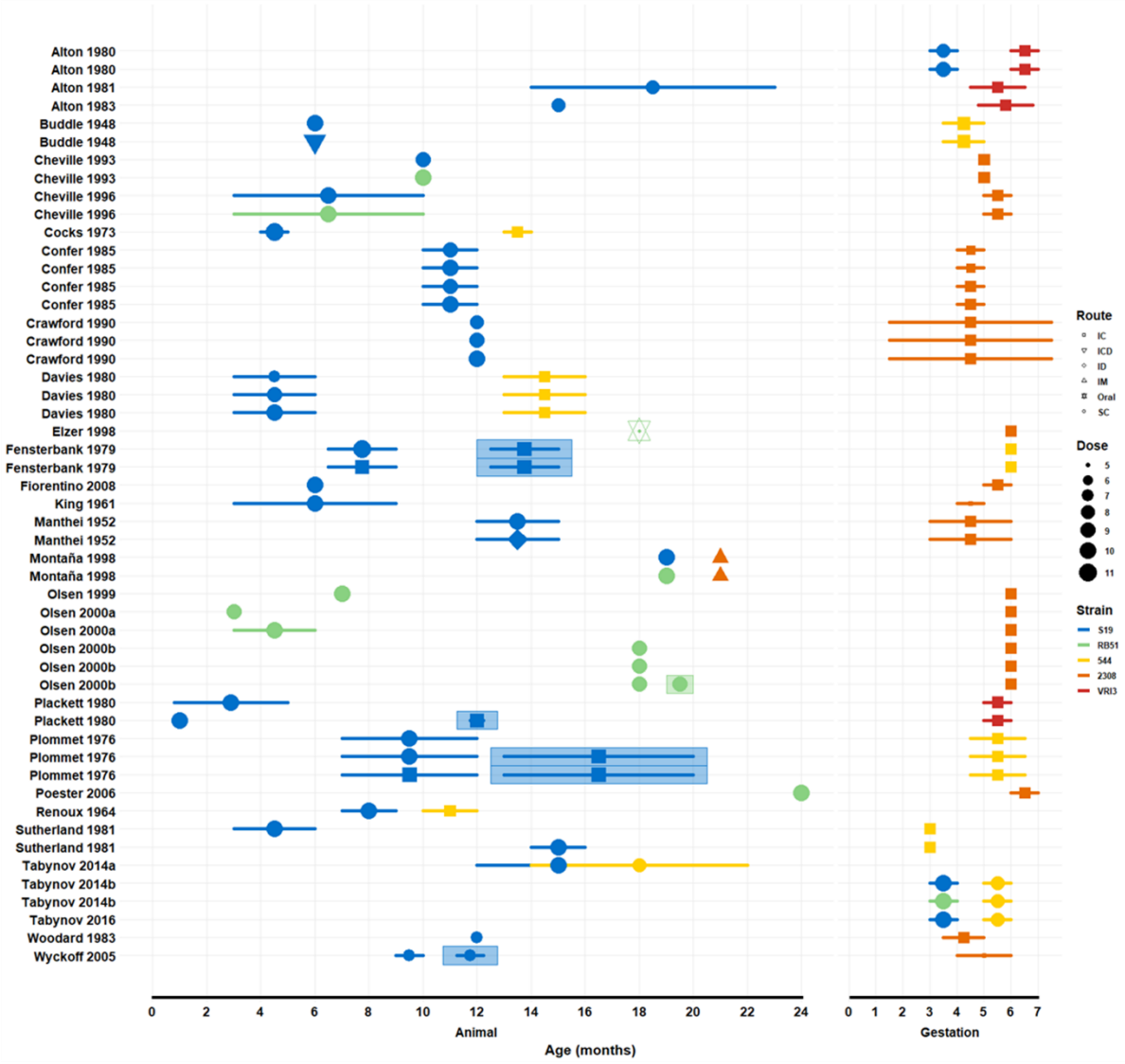
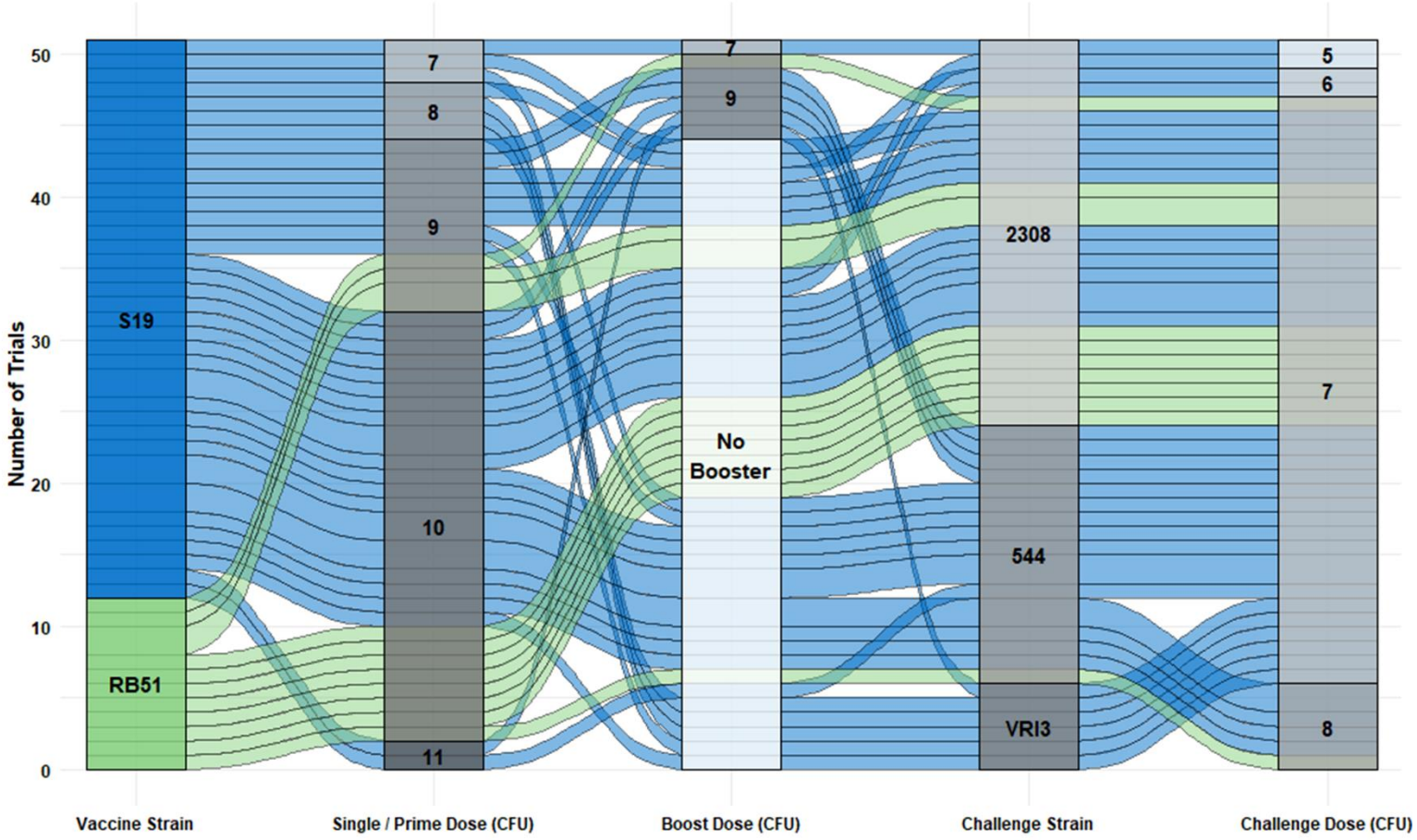


Figure 3 – Alluvial diagram showing the main experimental design characteristics of the 51 trials from 29 studies selected by this systematic review on the efficacy of bovine brucellosis vaccines.



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.

Table 2 – Results of post-vaccination brucellosis serological tests and clearance (antibodies and vaccine strain) according to the studies that performed these analyses among those selected by this systematic review.

First author, year	Vaccine dose	Age at vac[a]	Post-vaccination period of serological tests[b]	Serological tests	Clearance	
					Antib[c]	Strain
S19						
Alton, 1980[d]	5.6 x 10^9	3-4 preg[e]	UN[f]	RBT[g], CF[h], IHLT[i]	> 10 w	NT[j]
Alton, 1980[d]	2.8 x 10^8	3-4 preg	UN	RBT, CF, IHLT	> 10 w	NT
Alton, 1981	2.25 x 10^8	14-23 m[k]	UN	RBT, CF, IHLT	12 w	NT
Alton, 1983	3 x 10^8	15 m	UN	RBT, CF, IHLT	7 m	NT
Buddle, 1948	1.85 x 10^{10} SC[l]	6m	0, 2, 4, 9, 49 w[m]	STAT[n]	NT	NT
Buddle, 1948	1.85 x 10^{10} ICD[o]	6m	0, 2, 4, 9, 49 w	STAT	NT	NT
Cheville, 1993	3-10 x 10^9	10 m	0, 2, 4, 6 and 10 w	STAT	10 w	6 w
Cheville, 1996	1.31-1.71 x 10^{10}	3-10 m	0, 2, 4 and 10 w	STAT	NT[v]	12 w
Cocks, 1973	1.07 x 10^{11}	4-5 m	5, 7 and 10 d[p]; 4, 8 and 16 w	RBT, CF, STAT	> 28 w	NT
Crawford, 1990	1 x 10^8	12 m	1 to 9 m	RBT, CF, Riv[q], ELISA[r]	16 w	NT
Crawford, 1990	1 x 10^9	12 m	1 to 9 m	RBT, CF, Riv, ELISA	16 w	NT
Crawford, 1990	1 x 10^{10}	12 m	1 to 9 m	RBT, CF, Riv, ELISA	16 w	NT
Davies, 1980	9 x 10^7	3-6 m	-1, 0, 1 and 4 d; 1, 2, 3, 4, 5, 7, 9, 14, 17, 22, 27, 32, 36 and 40 w	RBT, CF, STAT	22 w	NT
Davies, 1980	4.5 x 10^9	3-6 m	-1, 0, 1 and 4 d; 1, 2, 3, 4, 5, 7, 9, 14, 17, 22, 27, 32, 36 and 40 w	RBT, CF, STAT	22 w	NT
Davies, 1980	9 x 10^{10}	3-6 m	-1, 0, 1 and 4 d; 1, 2, 3, 4, 5, 7, 9, 14, 17, 22, 27, 32, 36 and 40 w	RBT, CF, STAT	22 w	NT
Fensterbank, 1979	1.15 x 10^{11}/5.7 x 10^9	6.5-9 m / 12.5-15 m	1, 2, 3, and 4 w; 1 to 17 m	CF, STAT	58 w	NT
Fensterbank, 1979	6.1 x 10^9/5.7 x 10^9	6.5-9 m / 12.5-15 m	1, 2, 3, and 4 w; 1 to 17 m	CF, STAT	58 w	NT
Fiorentino, 2008	2 x 10^{10}	6 m	0 and 7 d; 2, 4, 6, 8, 10, and 12 w; 4 to17 m	RBT, CF, STAT, 2-ME[s], ELISA	> 8 w	NT
King, 1961	5 x 10^{10}	3-9 m	0, 7 and 14 d; 1 to 15 m	STAT	> 15 m	NT
Manthei, 1952	1.1-1.2 x 10^{10} SC	12-15 m	0, 5, 7, 10, 14, 16 and 18 d; 3, 4, 5, 6, 7, 8, 10, 12, 20, 28, 36, 44, 64 and 78 w	STAT	NT	NT
Manthei, 1952	1.1-1.2 x 10^{10} ID[t]	12-15 m	0, 5, 7, 10, 14, 16 and 18 d; 3, 4, 5, 6, 7, 8, 10, 12, 20, 28, 36, 44, 64 and 78 w	STAT	NT	NT
Montaña, 1998[t]	2 x 10^{10}	19 m	0, 8, 15, 30, 60 and 90 d	RBT, CF, RID[u], ELISA	NT	NT

Plackett, 1980	9 x 10^{10}	0.8-5 m	UN	RBT, CF, IHLT,	> 18 w	NT
Plackett, 1980	9 x 10^{10} /4.5 x 10^{9}	3-5 w / 12 m	UN	RBT, CF, IHLT,	> 18 w	NT
Plommet, 1976	9 x 10^{10}	7-12 m	0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 41 w	CF, STAT	29 w	NT
Plommet, 1976	9 x 10^{10} / 5 x 10^{9}	7-12 m / 13-20 m	0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, 29, 30, 31, 32, 34, 36, 37, 38, 39, 41 w	CF, STAT	29 w	NT
Plommet, 1976	5 x 10^{9} / 5 x 10^{9}	7-12 m / 13-20 m	0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, 29, 30, 31, 32, 34, 36, 37, 38, 39, 41 w	CF, STAT	29 w	NT
Woodard, 1983	5.9 x 10^{7}	12 m	UN	RBT, CF, Riv	< 5 m	NT
Wyckoff, 2005	1 x 10^{7} / 1 x 10^{7}	9-10 m / 11-13 m	-2, 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36 w	RBT, ELISA, PCFIA[v]	> 36 w	NT
RB51						
Cheville, 1993	1-1.4 x 10^{10}	10 m	0, 2, 4, 6, and 10 w	STAT, RB51 Dot blot	NT	6 w
Cheville, 1996	1-1.4 x 10^{10}	3-10 m	0, 2, 4, and 10 w	STAT, RB51 Dot blot	NT	12 w
Elzer, 1998	3 x 10^{10}	18 m	UN	RBT, RB51 Dot blot, ELISA	NT	NT
Montaña, 1998[t]	2 x 10^{10}	19 m	0, 8, 15, 30, 60 and 90 d	RBT, CF, RID, ELISA	NT	NT
Olsen, 1999	1.6-3.2 x 10^{10}	7 m	0, 2, 4, 6, and 10 w	STAT, RB51 Dot blot	NT	> 14 w
Olsen, 2000a	1.04 x 10^{9}	3 m	0, 4, 8, 12, and 16 w	STAT, RB51 Dot blot	12 w	NT
Olsen, 2000a	1.09-1.22 x 10^{10}	3-6 m	0, 4, 8, 12, and 16 w	STAT, RB51 Dot blot	16 w	NT
Olsen, 2000b	1 x 10^{9}	18 m	0, 4, 6, 10, 12, and 18 w	STAT, RB51 Dot blot	> 20 w	> 6 w
Olsen, 2000b	3 x 10^{9}	18 m	0, 4, 6, 10, 12, and 18 w	STAT, RB51 Dot blot	> 20 w	> 6 w
Olsen, 2000b	1 x 10^{9}/1 x 10^{9}	18 m / 19.5 m	0, 4, 6, 10, 12, and 18 w	STAT, RB51 Dot blot	> 20 w	> 6 w
Poester, 2006[x]	1.5 x 10^{10}	24 m	0, 15, 21, 30, 150, 270, 300, 360, and 380 d	RBT, STAT, 2-ME	NT	NT

[a]Age at vac: age at vaccination; [b]Post-vaccination period when serological tests were performed; [c]Antib: antibodies; [d]These trials used animals that were not in their first pregnancy; [e]preg: pregnancy; [f]UN: uninformed; [g]RBT: Rose Bengal Test; [h]CF: Complement Fixation Test; [i]IHLT: Indirect Hemolysis Test; [j]NT: not tested; [k]m: months; [l]SC: subcutaneous; [m]w: weeks; [n]STAT: Standard Tube Agglutination Test; [o]ICD: intracaudal; [p]d: days; [q]Riv: Rivanol; [r]ELISA: Enzyme Linked Immunossorbent Assay; [s]2-ME: 2-Mercaptoethanol; [t]ID: intradermal; [u]RID: Radial Immunodifusion; [v]PCFIA: Particle Concentration Fluorescence Immunoassay; [x]In this trial, 8 animals were vaccinated during early pregnancy. Buffered Plate Antigen, Acidified Plate and Card Test were grouped as Rose Bengal Test; The vaccine doses are in CFU (colony forming unit).

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 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.

Table 3 – Post-challenge serological results of brucellosis vaccinated and non-vaccinated animals according to trials that performed this analysis among those selected by this systematic review.

First author, year	Vaccine dose	Chal Dose[a]	Serological tests	Moment of testing	Positive serology	
					Vaccinated[b] (%)	Control[c] (%)
S19						
Cheville, 1993	3-10 x 10^9	1 x 10^7	STAT[d]	4, 8, 12, and 16 w[e]	5/6 (83)	4/5 (80)
Confer, 1985	1 x 10^9	9.4 x 10^6	RBT[f], CF[g], Riv[h]	0, 4, 8, and 14 w	1/11 (9)	5/9 (56)
Confer, 1985	1 x 10^{10}	9.4 x 10^6	RBT, CF, Riv	0, 4, 8, and 14 w	2/10 (20)	5/9 (56)
Confer, 1985	1 x 10^9	5.2 x 10^7	RBT, CF, Riv	0, 4, 8, and 14 w	8/11 (73)	6/9 (67)
Confer, 1985	1 x 10^{10}	5.2 x 10^7	RBT, CF, Riv	0, 4, 8, and 14 w	3/10 (30)	6/9 (67)
King, 1961	5 x 10^{10}	7.15-9 x 10^5	STAT	5 w	4/14 (29)	2/2 (100)
Manthei, 1952[i]	1.1-1.2 x 10^{10} SC[j]	1.6-2.6 x 10^7	STAT	1 to 18 w	19/20 (95)	31/31 (100)
Manthei, 1952[i]	1.1-1.2 x 10^{10} ID[k]	1.6-2.6 x 10^7	STAT	1 to 18 w	21/21 (100)	31/31 (100)
Wyckoff, 2005[l]	1 x 10^7 / 1 x 10^7	9.1 x 10^5	RBT, FI[m]	2 to 32 w	1/7 (14)	6/9 (67)
RB51						
Cheville, 1993	1-1.4 x 10^{10}	1 x 10^7	STAT	4, 8, 12, and 16 w	4/4 (100)	4/5 (80.00)
Elzer, 1998[l]	3 x 10^{10}	2 x 10^7	RBT	2 to 18 w	10/10 (100)	9/10 (90)
Olsen, 1999	1.6-3.2 x 10^{10}	1 x 10^7	STAT	2, 4, 6, and 10 w	12/12 (100)	6/6 (100)
Olsen, 2000a	1.04 x 10^9	1 x 10^7	STAT	4, 8, and 12 w	6/6 (100)	15/15 (100)
Olsen, 2000a	1.09-1.22 x 10^{10}	1 x 10^7	STAT	4, 8, and 12 w	26/26 (100)	15/15 (100)
Olsen, 2000b	1 x 10^9	1 x 10^7	STAT	4, 6, 10, 12, and 18 w	UN[n]	6/6 (100)
Olsen, 2000b	3 x 10^9	1 x 10^7	STAT	4, 6, 10, 12, and 18 w	UN	6/6 (100)
Olsen, 2000b	1 x 10^9 / 1 x 10^9	1 x 10^7	STAT	4, 6, 10, 12, and 18 w	UN	6/6 (100)
Poester, 2006[o]	1.5 x 10^{10}	3 x 10^7	STAT, RBT, 2-ME[p]	0, 15, 30, and 60 d[q]	11/20 (55)	11/13 (85)

[a]Chal. Dose: challenge dose; [b]Vaccinated: number of positive animals among those vaccinated; [c]Control: number of positive animals among control animals; [d]STAT: Standard Tube Agglutination Test; [e]w: weeks; [f]RBT: Rose Bengal Test; [g]CF: Complement Fixation Test; [h]Riv. Rivanol; [i]In these trials, the serological tests were performed weekly; [j]SC: subcutaneous; [k]ID: intradermal; [l]In these studies, the serological tests were performed once each two weeks; [m]FI: Fluorescence Immunoassay; [n]UN: uninformed; [o]In this trial, 8 animals were vaccinated during early pregnancy; [p]2-ME: 2-Mercaptoethanol; [q]d: days. The vaccine

and challenge doses are in CFU (colony forming unit). The cutoff point considered in all trial were those given by the authors and the test selected for the table were those with the highest number of positive animals

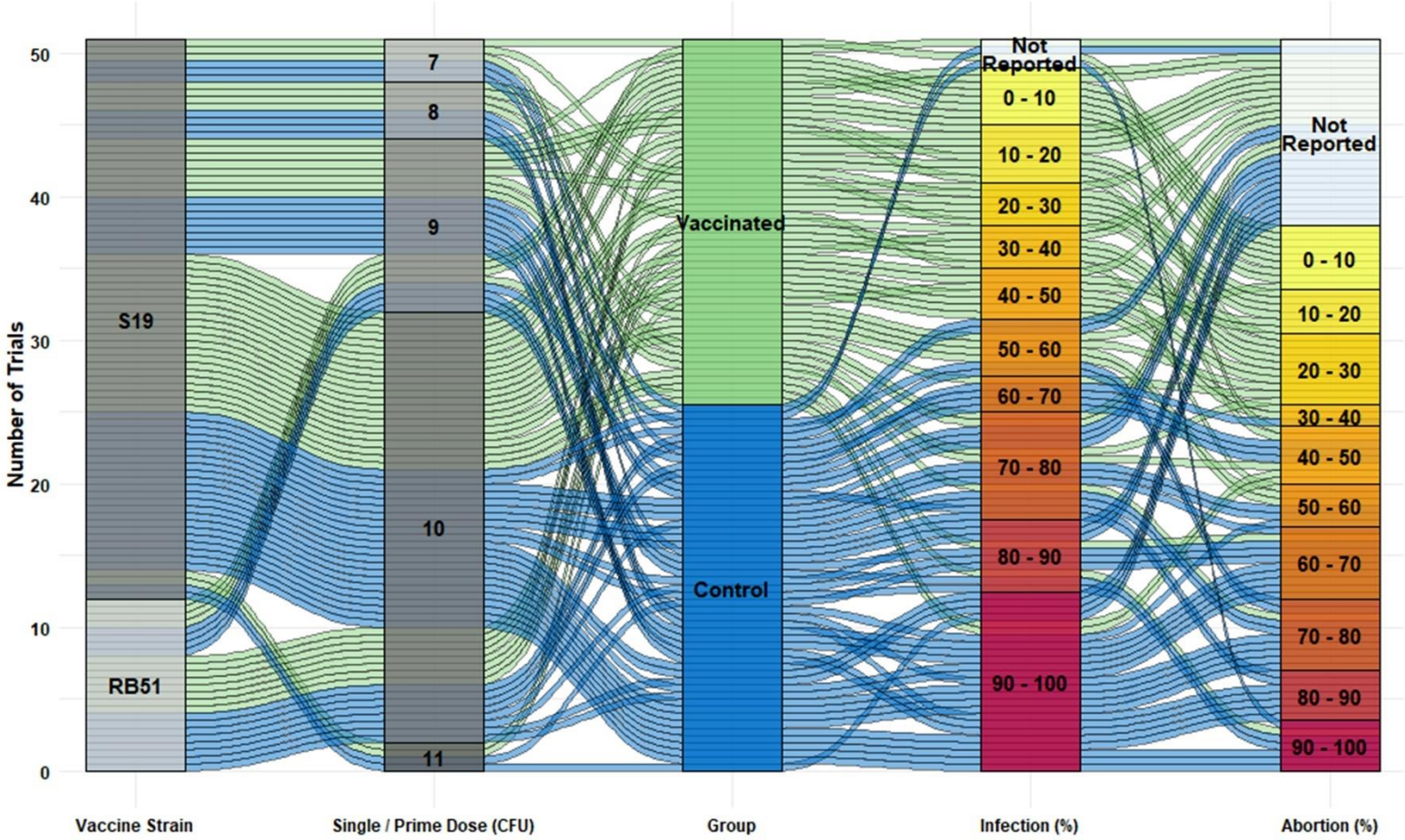
3.8- Assessment of protection against clinical signs

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.

Figure 4 – Alluvial diagram showing infection and abortion 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.



3.9- Assessment of protection against infection

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

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

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

3.10- Meta-analysis

For the meta-analysis regarding protection against reproductive clinical signs of brucellosis (grouped as abortion *lato sensu*), a total of 12 papers (15 trials) were selected and divided into 4 groups according to vaccine strain and dose used: S19 10^8 CFU / dose

(vaccinated with a dose close to 10^8 CFU of S19); S19 10^9 CFU / dose (vaccinated with a dose close to 10^9 CFU of S19); S19 10^{10} CFU / dose (vaccinated with a dose close to 10^{10} CFU of S19); and RB51 10^{10} CFU / dose (vaccinated with a dose close to 10^{10} CFU of RB51). In all these meta-analysis groups, animals were vaccinated subcutaneously, the challenge dose was close to or 1 x 10^7 CFU and all animals were exposed to *B. abortus* between 5 and 7 months of pregnancy. For the meta-analysis of protection against infection, a total of 17 papers (23 trials) were selected adding the group of non-pregnant animals vaccinated with S19 10^{10} CFU / dose and challenged with a dose close to or 1 x 10^7 CFU of virulent *B. abortus*. The RR and VE for abortion or *B. abortus* infection were the summary measures calculated. The meta-analysis results are shown in the Figure 5 and Figure 6.

Overall, the protection against abortion *lato sensu* in vaccinated animals was similar (RR = 0.41, 95% CI: 0.32 – 0.52; VE = 58.85%, 95% CI: 47.72 – 67.61) to protection against infection (RR = 0.43, 95% CI: 0.35 – 0.52; VE = 57.32%, 95% CI: 47.51 – 65.30) compared with non-vaccinated animals. The results of the meta-analysis showed that animals vaccinated with 10^{10} CFU of S19 have 1.89 times less probability to abort (RR = 0.53, 95% CI: 0.40 – 0.71; VE = 47.13%, 95% CI: 29.35 – 60.44) compared with animals in control groups. Animals vaccinated with 10^9 CFU of S19 exhibited 4 times less risk of abortion (RR = 0.25, 95% CI: 0.12 – 0.52; VE = 75.09%, 95% CI: 48.08 – 88.05) after challenge, than non-vaccinated animals. The probability of abortion after challenge was 2.5 (RR = 0.40, 95% CI: 0.21 – 0.75; VE = 60.00%, 95% CI: 25.02 – 78.66) times lower among vaccinated animals with 10^8 CFU of S19 compared with non-vaccinated ones. For meta-analysis of trials that used the RB51, animals that received the vaccine at the dose of 10^{10} CFU exhibited 3.23 (RR = 0.31, 95% CI: 0.16 – 0.61; VE = 69.25%, 95% CI: 39.48 – 84.38) times less probability of abortion after challenge, compared with non-vaccinated 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.

Figure 5 – Meta-analysis data and forest plot 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 *lato sensu*.

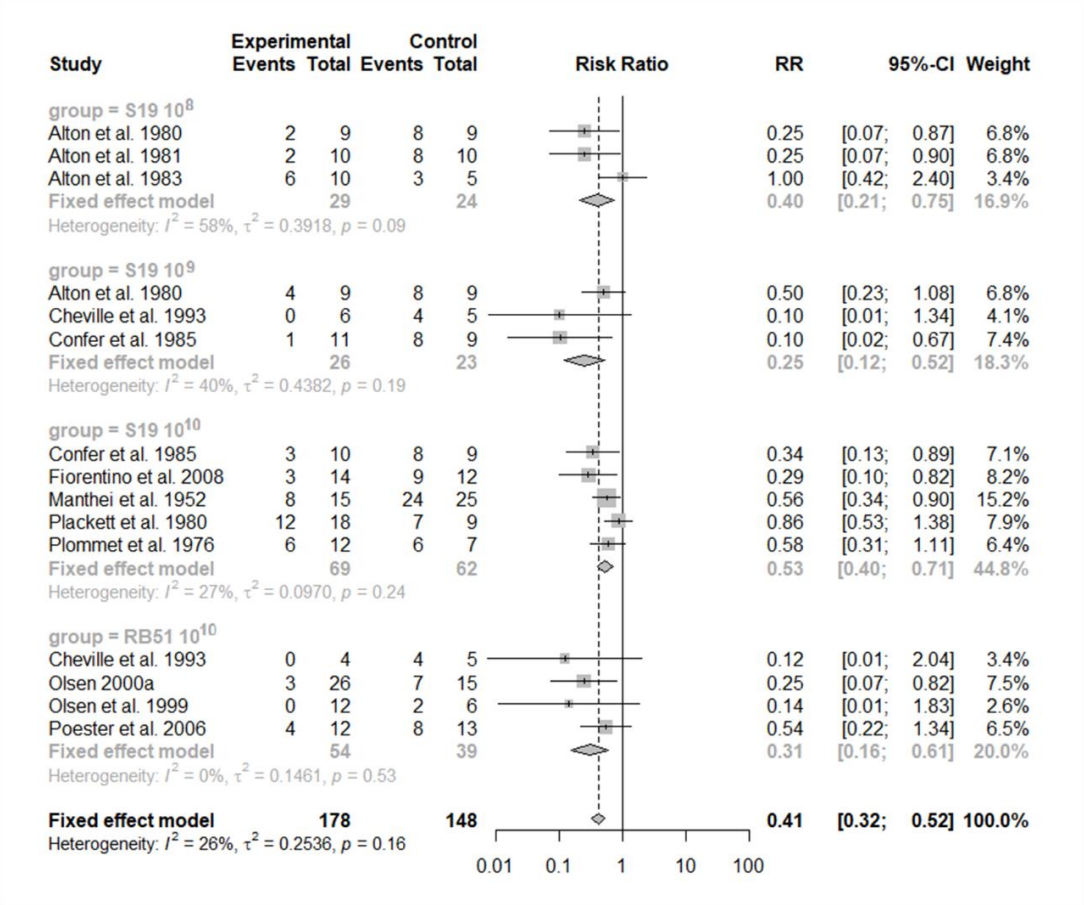


Figure 6 – Meta-analysis data and forest plot of protection against brucellosis infection after exposure to virulent *Brucella abortus* conferred by vaccination with S19 and RB51 at different doses. The data included the isolation of the challenge strain in any organ from the animals in the experiment,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

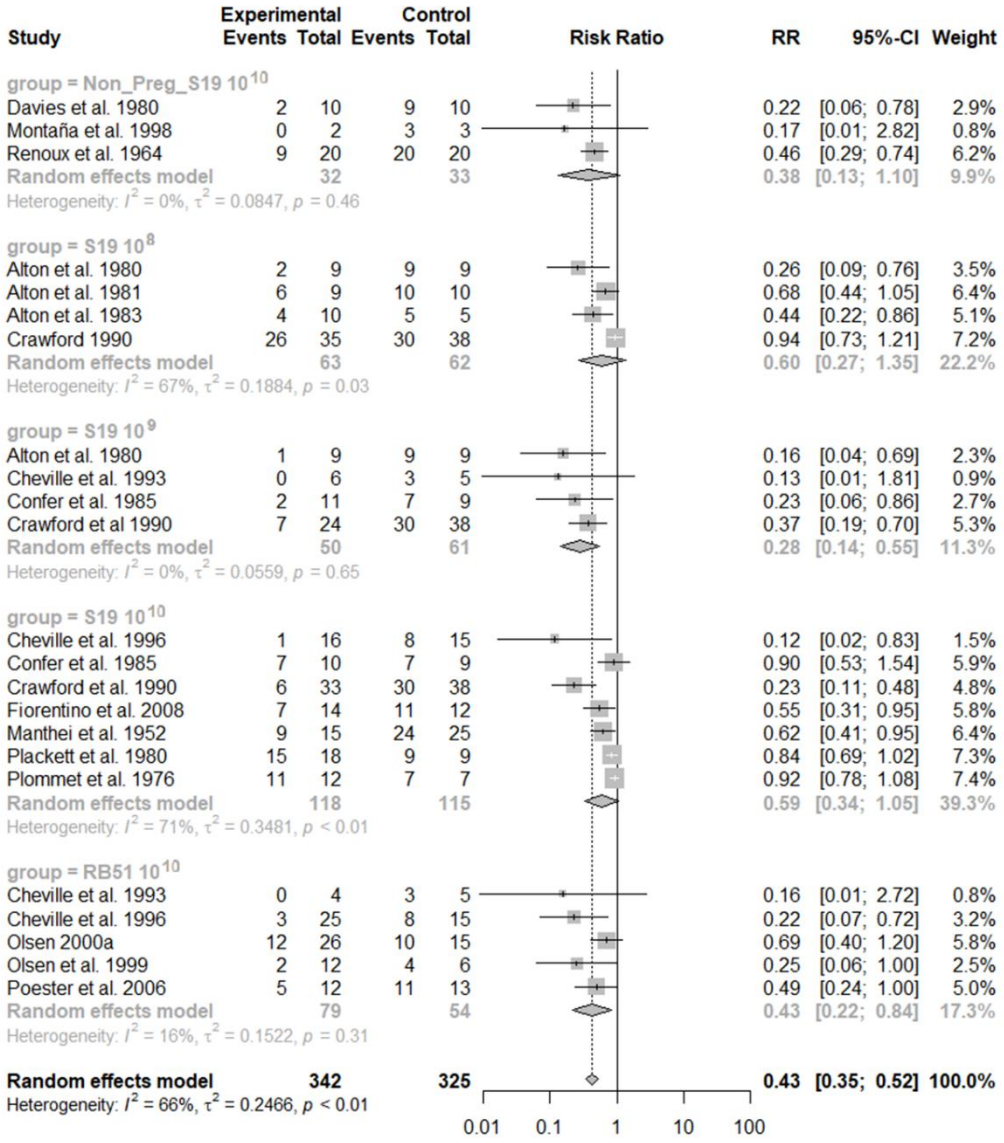
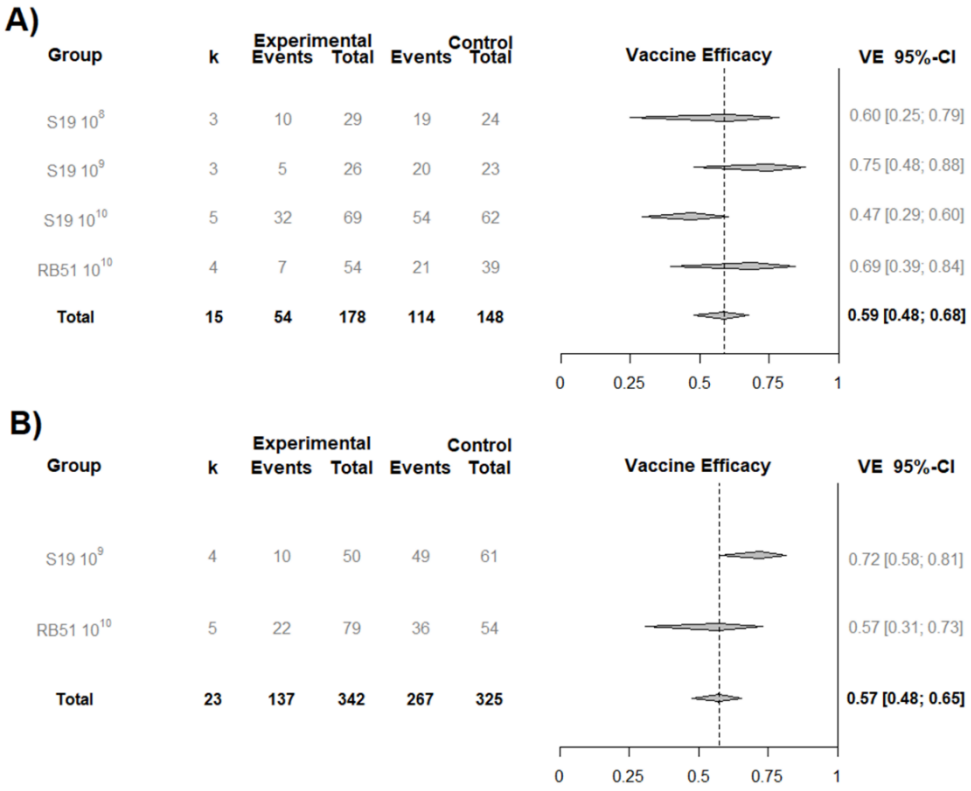


Figure 7 – Comparison of vaccine efficacy (VE) among meta-analysis subgroups for protection against abortion *lato sensu* (A) and infection (B) conferred by vaccination with S19 and RB51 at different doses after exposure to virulent *Brucella abortus*, for those subgroups that showed significant risk ratio. All reproductive clinical signs reported in the articles, as stillbirth, born of weak calves, premature calves and abortion were considered as abortion *lato sensu.* The data included the isolation of the challenge strain in any organ from the animals in the experiment, including fetal tissues. k – number of trials.



4- Discussion

This systematic review and meta-analysis aimed to analysis the efficacy of S19 and RB51 vaccines in high quality studies, from 1948 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 total 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 ≥ 1; Olsen et al. 2000b RR ≤ 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 3 and 6 months by the subcutaneous route is 5-8 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.

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 50-80 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 50-80 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 reemphasizes 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 (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 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 (a). 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, 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.

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 5×10^{10} CFU dose and the lowest challenge dose (9×10^5 CFU) among all trials, obtaining 28% seropositivity, and from Poester et al. (2006) that used RB51 vaccine at 1.5×10^{10} CFU dose and a challenge dose of 3×10^7 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 50-80 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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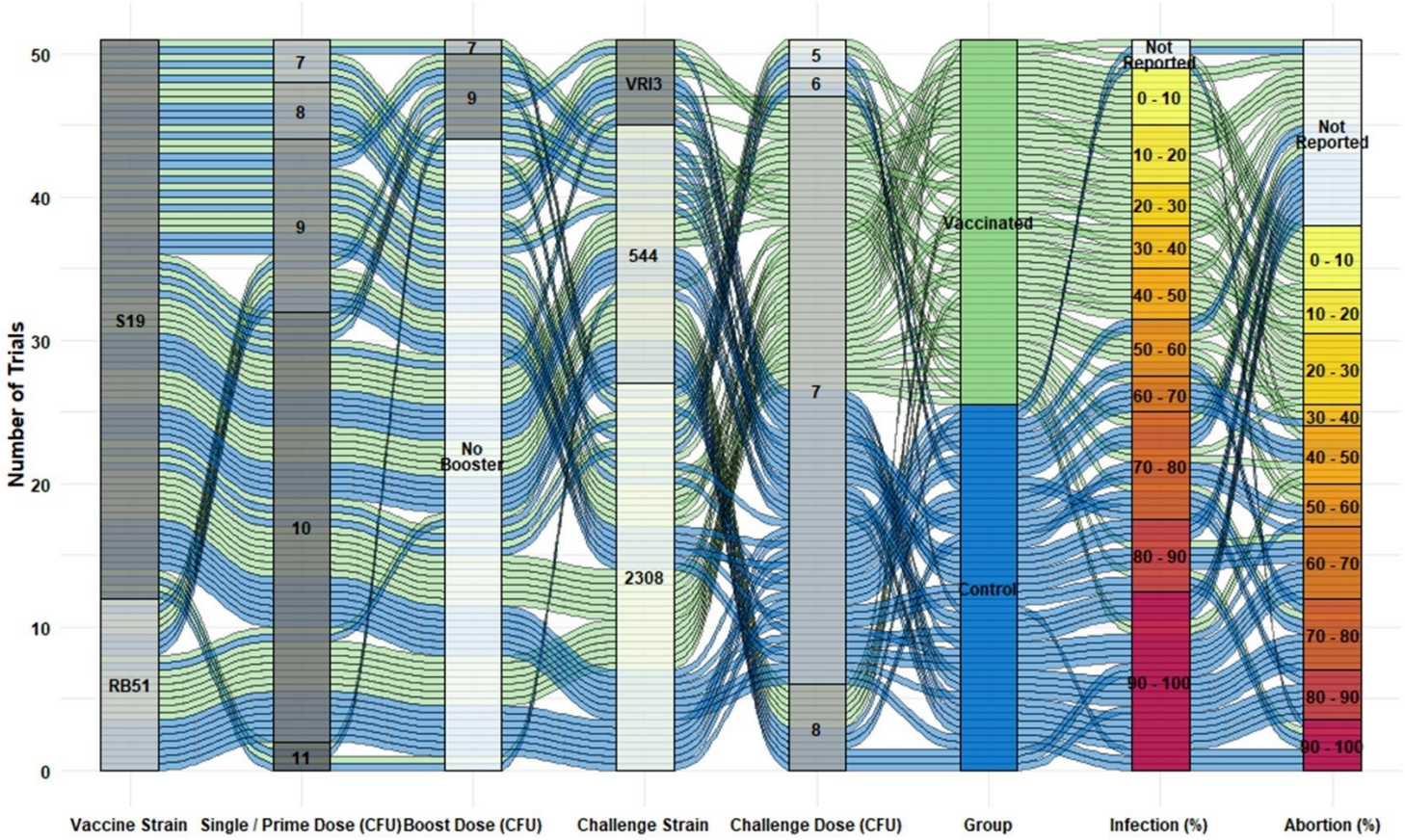
6 Supplementary Files

Supplementary Figure S1 – Alluvial diagram showing infection and abortion 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.



Supplementary Table S1 – Guidelines of PRISMA statement

Section/topic	#	Checklist item	Reported on §
		Title	
Title	1	Identify the report as a systematic review, meta-analysis, or both	§1
		Abstract	
Structured Summary	2	Provide s structured summary including, as aplicable: background; objectives; data sources; study elegibility criteria; participants and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number	§1
		Introduction	
Rationale	3	Describe the rationale for the review in the context of what is already known	§1, 2, 3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS)	§4
		Methods	
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	§2
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	§3, Tab.[1] S2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	§2
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	§Tab. S2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	§4 Tab. S3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators	§6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	§4-6 Tab. S2
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis	§6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	§7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis.	§7
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	§6
Additional analysis	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	§7
		Results	

Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	§1 Fig[2]. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Tab. S4
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	§1, 2 Fig. 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	§1 – 20 Tab. 1 – 5 Tab. S4
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	§21, 22 Fig. 2 - 4
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	-
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see item 16)]	§21, 22 Fig. 2 - 4
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers)	§1 - 2
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	§3 - 8
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	§9 - 11
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	§1

[1]Tab.: Table;[2]Fig.: Figure

Supplementary Table S2 – Search terms used in CABI, Cochrane, Pubmed, Scielo, Science Direct, Scopus and Web of Science databases, based on the PICOTS terms.

PICOTS	Search terms
Population	heifer* OR bovine* OR cattle OR cow* OR calf OR calve* OR pregnan*
Intervention	RB51 OR S19 OR SRB51 OR strain 19 OR strain RB51
Comparison	vaccin* OR challeng* OR efficacy OR experimen* OR infection AND brucel* AND abortus
Outcomes	immun* OR protect* OR safe* OR antibod*
Time	-
Setting	Systematic review and meta-analysis

Supplementary Table S3 – Inclusion and exclusion criteria for selection of articles in this systematic review.

Inclusion criteria	Exclusion criteria
• All countries • All years • *Brucella abortus* • Vaccination with S19 or RB51 • Challenge with virulent *B. abortus*	• Occupational brucellosis • Brucellosis caused by other species than *B. abortus* • Vaccination with other vaccines • No challenge performed • No information about clinical signs or bacteriology • Diagnostic performance of tests • Therapeutics • Genetics • Languages other than English, Spanish, French or Portuguese • Full-text not available • No information about age or pregnancy stage at challenge • No information about vaccine dose • No information about vaccine strain • No information about vaccine route • No information about challenge dose • No information about challenge strain • No information about challenge route • No information about clinical or infection protection

Supplementary Table S4 – Detailed relevant data of animal, vaccine, pregnancy and challenge of the trials selected by this systematic review.

First author, year	Cattle breed	Vaccination					Challenge			
		Strain	Age	Dose	N doses[a]	Volume	Age preg[b]	Met preg[c]	Route	Stage/Age
Alton, 1980[d]	Jersey	S19	3-4 preg[e]	5.6×10^9	1	2 mL	18-27 m[f]	NM[g]	IC[h]	6-7 preg
Alton, 1980[d]	Jersey	S19	3-4 preg	2.8×10^8	1	2 mL	18-27 m	NM	IC	6-7 preg
Alton, 1981	Jersey	S19	14-23 m	2.25×10^8	1	2 mL	18-27 m	NM	IC	4.5-6.5 preg
Alton, 1983	Jersey	S19	15 m	3×10^8	1	UN[i]	17 m	NM, AI[j]	IC	4.8-6.8 preg
Buddle, 1948	Jersey	S19	6 m	1.85×10^{10} SC[k]	1	5 mL	14-15.5 m	NM	IC	3.5-5 preg
Buddle, 1948	Jersey	S19	6 m	1.85×10^{10} ICD[l]	1	1 mL	14-15.5 m	NM	IC	3.5-5 preg
Cheville, 1993	Hereford	S19	10 m	$3\text{-}10 \times 10^9$	1	2 mL	16-19 m	NM	IC	5 preg
Cheville, 1993	Hereford	RB51	10 m	$1\text{-}1.4 \times 10^{10}$	1	4 mL	16-19 m	NM	IC	5 preg
Cheville, 1996	Hereford	S19	3-10 m	$1.31\text{-}1.71 \times 10^{10}$	1	4 mL	16-19 m	UN	IC	5-6 preg
Cheville, 1996	Hereford	RB51	3-10 m	$1\text{-}1.4 \times 10^{10}$	1	4 mL	16-19 m	UN	IC	5-6 preg
Cocks, 1973[m]	Crossbreed	S19	4-5 m	1.07×10^{11}	1	UN	NT[n]	NT	IC	13-14 m
Confer, 1985	Crossbreed	S19	10-12 m	1×10^9	1	2 mL	16-20 m	NM	IC	4-5 preg
Confer, 1985	Crossbreed	S19	10-12 m	1×10^{10}	1	2 mL	16-20 m	NM	IC	4-5 preg
Confer, 1985	Crossbreed	S19	10-12 m	1×10^9	1	2 mL	16-20 m	NM	IC	4-5 preg
Confer, 1985	Crossbreed	S19	10-12 m	1×10^{10}	1	2 mL	16-20 m	NM	IC	4-5 preg
Crawford, 1990	Crossbreed	S19	12 m	1×10^8	1	1 mL	UN	NM	IC	1.5-7.5 preg
Crawford, 1990	Crossbreed	S19	12 m	1×10^9	1	1 mL	UN	NM	IC	1.5-7.5 preg
Crawford, 1990	Crossbreed	S19	12 m	1×10^{10}	1	1 mL	UN	NM	IC	1.5-7.5 preg
Davies, 1980[m]	Jersey	S19	3-6 m	9×10^7	1	UN	NT	NT	IC	13-16 m
Davies, 1980[m]	Jersey	S19	3-6 m	4.5×10^9	1	UN	NT	NT	IC	13-16 m
Davies, 1980[m]	Jersey	S19	3-6 m	9×10^{10}	1	UN	NT	NT	IC	13-16 m
Elzer, 1998	UN	RB51	18 m	3×10^{10}	1	UN	24 m	NM	IC	6 preg
Fensterbank, 1979	Friesian[o]	S19	6.5-9 m / 12.5-15 m	1.15×10^{11} / 5.7×10^9	2	UN	14-16 m	AI	IC	6 preg
Fensterbank, 1979	Friesian[o]	S19	6.5-9 m / 12.5-15 m	$6.1 \times 10^9/5.7 \times 10^9$	2	UN	14-16 m	AI	IC	6 preg

Fiorentino, 2008	Crossbreed	S19	6 m	2×10^{10}	1	2 mL	17 m	NM	IC	5-6 preg
King, 1961	Holstein Friesian[o]	S19	3-9 m	5×10^{10}	1	UN	14-20 m	AI	IC	4-5 preg
Manthei, 1952	Jersey, Holstein	S19	12-15 m	$1.1\text{-}1.2 \times 10^{10}$ SC	1	0.2 and 5 mL	UN	UN	IC	3-6 preg
Manthei, 1952	Jersey, Holstein	S19	12-15 m	$1.1\text{-}1.2 \times 10^{10}$ ID[p]	1	0.2 mL	UN	UN	IC	3-6 preg
Montaña, 1998[m]	Criollo	S19	19 m	2×10^{10}	1	UN	NT	NT	IM[q]	21 m
Montaña, 1998	Criollo	RB51	19 m	2×10^{10}	1	UN	NT	NT	IM	21 m
Olsen, 1999	Hereford	RB51	7 m	$1.6\text{-}3.2 \times 10^{10}$	1	4 mL	24 m	NM	IC	6 preg
Olsen, 2000a	Hereford	RB51	3 m	1.04×10^{9}	1	2 mL	14-18 m	NM	IC	6 preg
Olsen, 2000a	Hereford	RB51	3-6 m	$1.09\text{-}1.22 \times 10^{10}$	1	2 mL	14-18 m	NM	IC	6 preg
Olsen, 2000b	Hereford	RB51	18 m	1×10^{9}	1	2 mL	18 m	NM	IC	6 preg
Olsen, 2000b	Hereford	RB51	18 m	3×10^{9}	1	2 mL	18 m	NM	IC	6 preg
Olsen, 2000b	Hereford	RB51	18 m / 19.5 m	1×10^{9} / 1×10^{9}	2	2 mL	18 m	NM	IC	6 preg
Plackett, 1980	UN	S19	0.8-5 m	9×10^{10}	1	2 mL	18-21 m	NM	IC	5-6 preg
Plackett, 1980	UN	S19	3-5 w[r] / 12 m	9×10^{10} / 4.5×10^{9}	2	2 mL / 0.1 mL	18-21 m	NM	IC	5-6 preg
Plommet, 1976	Frisonne[o]	S19	7-12 m	9×10^{10}	1	5 mL	15-20 m	AI	IC	4.5-6.5 preg
Plommet, 1976	Frisonne[o]	S19	7-12 m / 13-20 m	9×10^{10} / 5×10^{9}	2	5 mL/0.1 mL	15-20 m	AI	IC	4.5-6.5 preg
Plommet, 1976	Frisonne[o]	S19	7-12 m /13-20 m	5×10^{9} / 5×10^{9}	2	0.1 mL/0.1 mL	15-20 m	AI	IC	4.5-6.5 preg
Poester, 2006[s]	Crossbreed	RB51	24 m	1.5×10^{10}	1	2 mL	UN	AI	IC	6-7 preg
Renoux, 1964[m]	Limousine	S19	7-9 m	6×10^{10}	1	UN	NT	NT	IC	10-12 m
Sutherland, 1981	Crossbreed	S19	3-6 m	4×10^{10}	1	UN	14-18 m	NM	IC	3 preg
Sutherland, 1981	Crossbreed	S19	14-16 m	4×10^{10}	1	UN	14-18 m	NM	IC	3 preg
Tabynov, 2014a	UN	S19	12-18 m	8×10^{10}	1	2 mL	NT	UN	SC	14-22m
Tabynov, 2014b	Kazakh	S19	3-4 preg	8×10^{10}	1	UN	UN	AI	SC	5-6 preg
Tabynov, 2014b	Kazakh	RB51	3-4 preg	3.4×10^{10}	1	UN	UN	AI	SC	5-6 preg
Tabynov, 2016	Kazakh	S19	3-4 preg	8×10^{10}	1	UN	12-16 m	AI	SC	5-6 preg
Woodard, 1983	UN	S19	12 m	5.9×10^{7}	1	2 mL	12-14 m	NM	IC	3.5-5 preg
Wyckoff, 2005	Crossbreed	S19	9-10 m / 11-13 m	1×10^{7} / 1×10^{7}	2	2 mL	15-16 m	NM	IC	4-6 preg

[a]N dose: number of doses; [b]Age preg: age at pregnancy; [c]Met Preg: method of pregnancy used; [d]These trials used animals that were not in their first pregnancy; [e]Preg: pregnancy; [f]m: months; [g]NM: natural mating; [h]IC: intraconjunctival; [i]UN: uninformed; [j]AI: artificial insemination; [k]SC: subcutaneous; [l]ICD: intracaudal; [m]These trials challenge non-pregnant animals; [n]NT: not tested; [o]These breeds were grouped as Holstein breed in the manuscript; [p]ID: intradermal; [q]IM:intramuscular; [r]w: weeks; [s]In this trial, 8 animals were vaccinated during early pregnancy. The vaccine doses are in CFU (colony forming unit).

Supplementary Table S5 – Detailed information about the booster vaccination in the trials that performed this analysis among those selected by this systematic review.

First author, year	Strain	First vaccination			Booster vaccination			Challenge		Outcomes	
		Age	Dose	Route	Interval[a]	Dose	Route	Interval[b]	Dose	Abortion (%)	Infection (%)
Fenterbank, 1979	S19	6.5-9 m[c]	1.15×10^{11}	SC[d]	6 m	5.7×10^{9}	IC	14-16 m	1.48×10^{7}	3/22 (14)	10/22 (45)
Fenterbank, 1979	S19	6.5-9 m	6.1×10^{9}	IC[e]	6 m	5.7×10^{9}	IC	14-16 m	1.48×10^{7}	6/22 (27)	13/22 (59)
Olsen, 2000b	RB51	18 m	1×10^{9}	SC	6 w[f]	1×10^{9}	SC	25.5 m	1×10^{7}	0/4 (0)	0/4 (0)
Plackett, 1980	S19	3-5 w	9×10^{10}	SC	11 m	4.5×10^{9}	IC	7 -10 m	2×10^{7}	5/10 (50)	8/10 (80)
Plommet, 1976	S19	7-12 m	9×10^{10}	SC	6-8 m	5×10^{9}	IC	11.5-18.5 m	1.64×10^{7}	4/12 (33)	6/12 (50)
Plommet, 1976	S19	7-12 m	5×10^{9}	IC	6-8 m	5×10^{9}	IC	11.5-18.5 m	1.64×10^{7}	5/19 (26)	14/19 (74)
Wyckoff, 2005	S19	9-10 m	1×10^{7}	SC	9 w	1×10^{7}	SC	10.5 m	9.1×10^{5}	1/7 (14)	2/7 (29)

[a]Interval: interval between the vaccinations; [b]Interval: interval between the last vaccination and challenge; [c]m: months; [d]SC: subcutaneous; [e]IC: intra conjunctival; [f]w: weeks; The total number of reproductive outcomes was showed in the column "Abortion". The vaccine and challenge doses are in CFU (colony forming unit).

Supplementary Table S6 – Detailed data on the reproductive clinical signs of bovine brucellosis after challenge with virulent *Brucella abortus* according to trials that performed this analysis among those selected by this systematic review.

First author, year	Vaccine dose	Chal dose[a]	Abortion		Premature or weak calves		Stillbirth		Total outcomes	
			Vac[b] (%)	C[c] (%)	Vac (%)	C (%)	Vac (%)	C (%)	Vac (%)	C (%)
S19										
Alton, 1980[d]	2.8×10^8	2×10^7	0/9 (0)	5/9 (56)	1/9 (11)	3/9 (33)	1/9 (11)	0/9 (0)	2/9 (22)	8/9 (89)
Alton, 1980[d]	5.6×10^9	2×10^7	1/9 (11)	5/9 (56)	3/9 (33)	3/9 (33)	0/9 (0)	0/9 (0)	4/9 (44)	8/9 (89)
Alton, 1981	2.25×10^8	1.3×10^7	0/10 (0)	6/10 (60)	1/10 (10)	2/10 (20)	UN[e]	UN	1/10 (10)	8/10 (80)
Alton, 1983	3×10^8	1.3×10^7	1/10 (10)	3/5 (60)	3/10 (30)	0/5 (0)	2/10 (20)	0/5 (0)	6/10 (60)	3/5 (60)
Buddle, 1948	1.85×10^{10}-SC[f]	1.7×10^8	12/48 (25)	24/44 (55)	UN	UN	UN	UN	12/48 (25)	24/44 (55)
Buddle, 1948	1.85×10^{10} ICD[g]	1.7×10^8	15/42 (36)	24/44 (55)	UN	UN	UN	UN	15/42 (36)	24/44 (55)
Cheville, 1993	$3\text{-}10 \times 10^9$	1×10^7	0/6 (0)	4/5 (80)	UN	UN	UN	UN	0/6 (0)	4/5 (80)
Confer, 1985	1×10^9	9.4×10^6	UN[e]	UN	UN	UN	UN	UN	1/11 (9)	8/9 (89)
Confer, 1985	1×10^{10}	9.4×10^6	UN	UN	UN	UN	UN	UN	3/10 (30)	8/9 (89)
Confer, 1985	1×10^9	5.2×10^7	UN	UN	UN	UN	UN	UN	8/10 (80)	9/9 (100)
Confer, 1985	1×10^{10}	5.2×10^7	UN	UN	UN	UN	UN	UN	8/8 (100)	9/9 (100)
Fensterbank, 1979	$1.15 \times 10^{11} / 5.7 \times 10^9$	1.48×10^7	3/22 (14)	3/6 (50)	0/22 (0)	1/6 (17)	UN	UN	3/22 (14)	4/6 (67)
Fensterbank, 1979	$6.1 \times 10^9 / 5.7 \times 10^9$	1.48×10^7	3/22 (14)	3/6 (50)	3/22 (14)	1/6 (17)	UN	UN	6/22 (27)	4/6 (67)
Fiorentino, 2008	2×10^{10}	3×10^7	UN	UN	UN	UN	UN	UN	3/14 (21)	9/12 (75)
King, 1961	5×10^{10}	$7.15\text{-}9 \times 10^5$	UN	UN	UN	UN	UN	UN	3/14 (21)	2/2 (100)
Manthei, 1952	$1.1\text{-}1.2 \times 10^{10}$ SC	$1.6\text{-}2.6 \times 10^7$	6/17 (35)	25/30 (83)	3/17 (18)	4/30 (13)	UN	UN	9/17 (53)	29/30 (97)
Manthei, 1952	$1.1\text{-}1.2 \times 10^{10}$ ID[h]	$1.6\text{-}2.6 \times 10^7$	8/21 (38)	25/30 (83)	4/21 (19)	4/30 (13)	UN	UN	12/21 (57)	29/30 (97)
Plackett, 1980	9×10^{10}	2×10^7	UN	UN	UN	UN	UN	UN	12/18 (67)	7/9 (78)
Plackett, 1980	$9 \times 10^{10} / 4.5 \times 10^9$	2×10^7	UN	UN	UN	UN	UN	UN	5/10 (50)	7/9 (78)
Plommet, 1976	9×10^{10}	1.64×10^7	4/12 (33)	5/7 (71)	2/12 (17)	1/7 (14)	UN	UN	6/12 (50)	6/7 (86)
Plommet, 1976	$9 \times 10^{10} / 5 \times 10^9$	1.64×10^7	4/12 (33)	5/7 (71)	0/12 (0)	1/7 (14)	UN	UN	4/12 (33)	6/7 (86)
Plommet, 1976	$5 \times 10^9 / 5 \times 10^9$	1.64×10^7	3/19 (16)	5/7 (71)	2/19 (11)	1/7 (14)	UN	UN	5/19 (26)	6/7 (86)
Sutherland, 1981	4×10^{10} (3-6 m[i])	1×10^7	1/7 (14)	5/8 (63)	0/7 (0)	1/8 (13)	0/7 (0)	0/8 (0)	1/7 (14)	6/8 (75)
Sutherland, 1981	4×10^{10} (14-16 m)	1×10^7	0/11 (0)	5/8 (63)	0/11 (0)	1/8 (13)	0/11 (0)	0/8 (0)	0/11 (0)	6/8 (75)

Tabynov, 2014b	8 x 10^{10}	5 x 10^{8}	UN	UN	UN	UN	UN	UN	1/9 (11)	7/10 (70)
Tabynov, 2016	8 x 10^{10}	5 x 10^{8}	UN	UN	UN	UN	UN	UN	1/8 (13)	5/7 (71)
Woodard, 1983	5.9 x 10^{7}	2.55 x 10^{7}	UN	UN	UN	UN	UN	UN	6/12 (50)	17/18 (94)
Wyckoff, 2005	1 x 10^{7} / 1 x 10^{7}	9.1 x 10^{5}	UN	UN	UN	UN	UN	UN	1/7 (14)	4/9 (44)
RB51										
Cheville, 1993	1-1.4 x 10^{10}	1 x 10^{7}	0/4 (0)	3/5 (60)	0/4 (0)	1/5 (20)	UN	UN	0/4 (0)	4/5 (80)
Elzer, 1998	3 x 10^{10}	2 x 10^{7}	UN	UN	UN	UN	UN	UN	3/10 (30)	7/10 (70)
Olsen, 1999	1.6 – 3.2 x 10^{10}	1 x 10^{7}	UN	UN	0/12 (0)	2/6 (33)	UN	UN	0/12 (0)	2/6 (33)
Olsen, 2000a	1.04 x 10^{9}	1 x 10^{7}	UN	UN	UN	UN	UN	UN	2/4 (50)	7/15 (47)
Olsen, 2000a	1.09-1.22 x 10^{10}	1 x 10^{7}	UN	UN	UN	UN	UN	UN	3/26 (12)	7/15 (47)
Olsen, 2000b	1 x 10^{9}	1 x 10^{7}	UN	UN	UN	UN	UN	UN	0/7 (0)	4/6 (67)
Olsen, 2000b	3 x 10^{9}	1 x 10^{7}	UN	UN	UN	UN	UN	UN	0/4 (0)	4/6 (67)
Olsen, 2000b	1 x 10^{9}/1 x 10^{9}	1 x 10^{7}	UN	UN	UN	UN	UN	UN	0/4 (0)	4/6 (67)
Poester, 2006j	1.5 x 10^{10}	3 x 10^{7}	UN	UN	UN	UN	UN	UN	5/20 (25)	8/13 (61)
Tabynov, 2014b	3.4 x 10^{10}	5 x 10^{8}	UN	UN	UN	UN	UN	UN	3/10 (30)	7/10 (70)

[a]Chal dose: challenge dose; [b]Vac: number of outcomes among vaccinated animals; [c]C: number of outcomes among control animals; [d]These trials used animals that were not in their first pregnancy; [e]UN: uninformed; [f]SC: subcutaneous; [g]ICD: intracaudal; [h]ID: Intradermal; [i]m: months; [j]In this trial, 8 animals were vaccinated during early pregnancy. The vaccine and challenge doses are in CFU (colony forming unit). When authors defined abortion in broader way covering other reproductive outcomes, the total number of outcomes were showed in column "Total outcomes".

Supplementary Table S7 – Data on the stage of pregnancy at challenge and the gestational age of abortion according to trials that performed this analysis among those selected by this systematic review.

First author, year	Vaccination		Challenge		Abortion		Gestational age (days)	
	Strain	Dose	Dose	Preg stage[a] (m)[b]	Vaccinated (%)	Control (%)	Mean, ±[c]	Median, IQR[d]
Confer, 1985	S19	1 x 10^9	9.4 x 10^6	4-5	1/11 (9)	8/9 (89)	251.8 ± 19.6	UN[e]
Confer, 1985	S19	1 x 10^{10}	9.4 x 10^6	4-5	3/10 (30)	8/9 (89)	245.2 ± 12.8	UN
Confer, 1985	S19	1 x 10^9	5.2 x 10^7	4-5	8/10 (80)	9/9 (100)	204.4 ± 19.5	UN
Confer, 1985	S19	1 x 10^{10}	5.2 x 10^7	4-5	8/8 (100)	9/9 (100)	202.1 ± 11.5	UN
Fensterbank, 1979	S19	1.15 x 10^{11} / 5.7 x 10^9	1.48 x 10^7	6	3/22 (14)	4/6 (67)	271 ± 18	277, 271 – 281
Fensterbank, 1979	S19	6.1 x 10^9 / 5.7 x 10^9	1.48 x 10^7	6	6/22 (27)	4/6 (67)	272 ± 14	276, 262 – 282
King, 1961	S19	5 x 10^{10}	7.15-9 x 10^5	4-5	3/14 (21)	2/2 (100)	263 ± 35	278, 267 – 280
Manthei, 1952	S19	1.1-1.2 x 10^{10} SC[f]	1.6-2.6 x 10^7	3-6	9/17 (53)	29/30 (97)	267 ± 22	279, 253 – 284
Manthei, 1952	S19	1.1-1.2 x 10^{10} ID[g]	1.6-2.6 x 10^7	3-6	12/21 (57)	29/30 (97)	262 ± 21	273, 242 – 280
Plommet, 1976	S19	9 x 10^{10}	1.64 x 10^7	4.5-6.5	6/12 (50)	6/7 (86)	260 ± 15	262, 250 – 270
Plommet, 1976	S19	9 x 10^{10} / 5 x 10^9	1.64 x 10^7	4.5-6.5	4/12 (33)	6/7 (86)	264 ± 20	271, 256 – 276
Plommet, 1976	S19	5 x 10^9 / 5 x 10^9	1.64 x 10^7	4.5-6.5	5/19 (26)	6/7 (86)	269 ±12	272, 259 – 277
Woodard, 1983	S19	5.9 x 10^7	2.55 x 10^7	3.5-5	6/12 (50)	17/18 (94)	205 ± 68	UN

[a]Preg stage: pregnancy stage; [b]m: months; [c]±: standard deviation; [d]IQR: interquartile range; [e]UN: uniformed; [f]SC: subcutaneous; [g]ID: intradermal. The total number of reproductive outcomes was showed in the column "Abortion". The vaccine and challenge doses are in CFU (colony forming unit).

Supplementary Table S8 – Detailed data on protection against infection after challenge with virulent *Brucella abortus* according to trials that performed this analysis among those selected by this systematic review.

First author, year	Vac dose[a]	Chal dose[b]	Foetal membrane or placenta		Foetus or calf		Colostrum or milk		Vag excret or uterus[e]		Lymph nodes		Total mat bct[f]		Total bacteriology	
			Vac[c] (%)	C[d] (%)	Vac (%)	C (%)	Vac (%)	C (%)	Vac (%)	C (%)	Vac (%)	C (%)	Vac (%)	C (%)	Vac (%)	C (%)
S19																
Alton, 1980[g]	2.8×10^8	2×10^7	2/9 (22)	9/9 (100)	1/9 (11)	6/9 (66)	2/9 (22)	9/9 (100)	1/9 (11)	8/8 (100)	UN[h]	UN	2/9 (22)	9/9 (100)	2/9 (22)	9/9 (100)
Alton, 1980[g]	5.6×10^9	2×10^7	1/9 (11)	9/9 (100)	1/9 (11)	6/9 (66)	1/9 (11)	9/9 (100)	1/8 (13)	8/8 (100)	UN	UN	1/9 (11)	9/9 (100)	1/9 (11)	9/9 (100)
Alton, 1981	2.25×10^8	1.3×10^7	5/9 (56)	10/10 (100)	2/9 (22)	10/10 (100)	5/9 (56)	10/10 (100)	4/8 (50)	7/9 (78)	UN	UN	6/9 (67)	10/10 (100)	6/10 (60)	10/10 (100)
Alton, 1983	3×10^8	1.3×10^7	4/10 (40)	4/5 (80)	4/10 (40)	4/5 (80)	4/10 (40)	5/5 (100)	3/10 (30)	3/5 (60)	UN	UN	4/10 (40)	5/5 (100)	4/10 (40)	5/5 (100)
Buddle, 1948	1.85×10^{10}-SC[i]	1.7×10^8	UN	UN	UN	UN	17/48 (35)	31/44 (71)	UN	UN	UN	UN	17/48 (35)	31/44 (71)	17/48 (35)	31/44 (71)
Buddle, 1948	1.85×10^{10} ICD[j]	1.7×10^8	UN	UN	UN	UN	18/42 (43)	31/44 (71)	UN	UN	UN	UN	18/42 (43)	31/44 (71)	18/42 (43)	31/44 (71)
Cheville, 1993	$3\text{-}10 \times 10^9$	1×10^7	0/6 (0)	2/5 (40)	0/6 (0)	3/5 (60)	0/6 (0)	3/5 (60)	0/6 (0)	1/5 (20)	0/6 (0)	2/5 (40)	0/6 (0)	3/5 (60)	0/6 (0)	3/5 (60)
Cheville, 1996	$1.31\text{-}1.71 \times 10^{10}$	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	1/16 (6)	8/15 (53)
Cocks, 1973[k]	1.07×10^{11}	2.15×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	4/11 (36)	10/11 (91)
Confer, 1985	1×10^9	9.4×10^6	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	2/11 (18)	7/9 (78)
Confer, 1985	1×10^{10}	9.4×10^6	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	7/10 (70)	7/9 (78)
Confer, 1985	1×10^9	5.2×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	8/10 (80)	8/9 (89)
Confer, 1985	1×10^{10}	5.2×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	7/8 (88)	8/9 (89)
Crawford, 1990	1×10^8	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	26/35 (74)	30/38 (79)
Crawford, 1990	1×10^9	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	7/24 (29)	30/38 (79)
Crawford, 1990	1×10^{10}	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	6/33 (19)	30/38 (79)
Davies, 1980[k]	9×10^7	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	8/10 (80)	9/10 (90)	UN	UN	8/10 (80)	9/10 (90)
Davies, 1980[k]	4.5×10^9	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	1/9 (11)	9/10 (90)	UN	UN	1/9 (11)	9/10 (90)
Davies, 1980[k]	9×10^{10}	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	2/10 (20)	9/10 (90)	UN	UN	2/10 (20)	9/10 (90)
Fensterbank, 1979	$1.15 \times 10^{11}/5.7 \times 10^9$	1.48×10^7	UN	UN	2/2 (100)	4/4 (100)	9/22 (41)	5/6 (83)	8/22 (36)	5/6 (83)	11/22 (50)	4/6 (67)	10/22 (45)	5/6 (83)	10/22 (45)	5/6 (83)
Fensterbank, 1979	$6.1 \times 10^9/5.7 \times 10^9$	1.48×10^7	UN	UN	3/3 (100)	4/4 (100)	12/22 (55)	5/6 (83)	9/22 (41)	5/6 (83)	13/22 (59)	4/6 (67)	13/22 (59)	5/6 (83)	13/22 (59)	5/6 (83)
Fiorentino, 2008	2×10^{10}	3×10^7	1/5 (20)	9/10 (90)	1/3 (33)	9/9 (100)	3/14 (21)	9/12 (75)	6/14 (43)	11/12 (92)	UN	UN	7/14 (50)	11/12 (92)	7/14 (50)	11/12 (92)
King, 1961	5×10^{10}	$7.15\text{-}9 \times 10^5$	UN	UN	2/3 (67)	2/2 (100)	5/14 (36)	2/2 (100)	3/14 (21)	2/2 (100)	UN	UN	5/14 (36)	2/2 (100)	5/14 (36)	2/2 (100)
Manthei, 1952	$1.1\text{-}1.2 \times 10^{10}$ SC	$1.6\text{-}2.6 \times 10^7$	UN	UN	8/9 (89)	27/29 (93)	9/19 (47)	30/31 (97)	8/17 (47)	29/30 (97)	UN	UN	9/19 (47)	30/31 (97)	10/19 (53)	30/31 (97)
Manthei, 1952	$1.1\text{-}1.2 \times 10^{10}$ ID[l]	$1.6\text{-}2.6 \times 10^7$	UN	UN	7/8 (86)	27/29 (93)	8/21 (38)	30/31 (97)	11/21 (52)	29/30 (97)	UN	UN	12/21 (57)	30/31 (97)	12/21 (57)	30/31 (97)
Montaña, 1998[k]	2×10^{10}	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	0/2 (0)	3/3 (100)
Plackett, 1980	9×10^{10}	2×10^7	13/17 (76)	8/8 (100)	14/17 (82)	9/9 (100)	16/18 (89)	9/9 (100)	UN	UN	UN	UN	16/18 (89)	9/9 (100)	16/18 (89)	9/9 (100)
Plackett, 1980	$9 \times 10^{10}/4.5 \times 10^9$	2×10^7	7/9 (78)	8/8 (100)	4/6 (67)	9/9 (100)	8/10 (80)	9/9 (100)	UN	UN	UN	UN	8/10 (80)	9/9 (100)	8/10 (80)	9/9 (100)
Plommet, 1976	9×10^{10}	1.64×10^7	UN	UN	4/4 (100)	5/5 (100)	9/12 (75)	7/7 (100)	11/12 (92)	7/7 (100)	9/12 (75)	7/7 (100)	11/12 (92)	7/7 (100)	11/12 (92)	7/7 (100)
Plommet, 1976	$9 \times 10^{10}/5 \times 10^9$	1.64×10^7	UN	UN	3/4 (75)	5/5 (100)	4/12 (33)	7/7 (100	6/12 (50)	7/7 (100)	4/12 (33)	7/7 (100)	6/12 (50)	7/7 (100)	6/12 (50)	7/7 (100)
Plommet, 1976	$5 \times 10^9/5 \times 10^9$	1.64×10^7	UN	UN	3/4 (75)	5/5 (100)	12/17 (71)	7/7 (100	10/19 (53)	7/7 (100)	11/19 (58)	7/7 (100)	14/19 (74)	7/7 (100)	14/19 (74)	7/7 (100)
Renoux, 1964[k]	6×10^{10}	1.5×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	9/20 (45)	20/20 (100)
Sutherland, 1981	4×10^{10} (3-6 m[m])	1×10^7	UN	UN	0/6 (0)	2/4 (50)	2/7 (29)	6/8 (75)	UN	UN	UN	UN	UN	UN	2/7 (29)	6/8 (75)
Sutherland, 1981	4×10^{10} (14-16 m)	1×10^7	UN	UN	0/11 (0)	2/4 (50)	0/11 (0)	6/8 (75)	UN	UN	UN	UN	UN	UN	0/11 (0)	6/8 (75)
Tabynov, 2014b	8×10^{10}	5×10^8	UN	UN	1/9 (11)	10/10 (100)	UN	UN	UN	UN	UN	UN	2/9 (22)	10/10 (100)	2/9 (22)	10/10 (100)
Tabynov, 2016	8×10^{10}	5×10^8	UN	UN	1/8 (13)	6/7 (86)	UN	UN	UN	UN	UN	UN	2/8 (25)	7/7 (100)	2/8 (25)	7/7 (100)
Wyckoff, 2005	1×10^7 / 1×10^7	9.1×10^5	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	2/7 (29)	6/9 (67)
RB51																
Cheville, 1993	$1\text{-}1.4 \times 10^{10}$	1×10^7	0/6 (0)	2/5 (40)	0/6 (0)	3/5 (60)	0/6 (0)	3/5 (60)	0/6 (0)	1/5 (20)	0/6 (0)	2/5 (40)	0/6 (0)	3/5 (60)	0/6 (0)	3/5 (60)
Cheville, 1996	$1\text{-}1.4 \times 10^{10}$	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	3/25 (12)	8/15 (53)
Elzer, 1998	3×10^{10}	2×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	2/10 (20)	8/10 (80)
Montaña, 1998	2×10^{10}	1×10^7	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	1/3 (33)	3/3 (100)

Olsen, 1999	1.6-3.2 x 10^{10}	1 x 10^{7}	0/12 (0)	0/4 (0)	0/12 (0)	1/6 (17)	1/12 (8)	3/6 (50)	0/12 (0)	2/6 (33)	1/9 (11)	1/1(100)	2/12 (17)	4/6 (67)	2/12 (17)	4/6 (67)
Olsen 2000a	1.04 x 10^{9}	1 x 10^{7}	UN	UN	2/4 (50)	7/15 (47)	UN	UN	UN	UN	UN	UN	4/4 (100)	10/15 (67)	4/4 (100)	10/15 (67)
Olsen 2000a	1.09-1.22 x 10^{10}	1 x 10^{7}	UN	UN	3/26 (12)	7/15 (47)	UN	UN	UN	UN	UN	UN	12/26 (46)	10/15 (67)	12/26 (46)	10/15 (67)
Olsen, 2000b	1 x 10^{9}	1 x 10^{7}	UN	UN	0/7 (0)	4/6 (67)	UN	UN	UN	UN	UN	UN	0/7 (0)	6/6 (100)	0/7 (0)	6/6 (100)
Olsen, 2000b	3 x 10^{9}	1 x 10^{7}	UN	UN	0/4 (0)	4/6 (67)	UN	UN	UN	UN	UN	UN	0/4 (0)	6/6 (100)	0/4 (0)	6/6 (100)
Olsen, 2000b	1 x 10^{9}/1 x 10^{9}	1 x 10^{7}	UN	UN	0/4 (0)	4/6 (67)	UN	UN	UN	UN	UN	UN	0/4 (0)	6/6 (100)	0/4 (0)	6/6 (100)
Poester, 2006[n]	1.5 x 10^{10}	3 x 10^{7}	7/20 (35)	11/13 (85)	6/20 (30)	9/13 (69)	4/20 (20)	10/13 (77)	6/20 (30)	11/13 (85)	3/20 (15)	9/13 (69)	7/20 (35)	11/13 (85)	7/20 (35)	11/13 (85)
Tabynov, 2014b	3.4 x 10^{10}	5 x 10^{8}	UN	UN	4/10 (40)	10/10 (100)	UN	UN	UN	UN	UN	UN	5/10 (50)	10/10 (100)	5/10 (50)	10/10 (100)

[a]Vac dose: vaccine dose; [b]Chal dose: challenge dose; [c]Vac: number of outcomes among those vaccinated; [d]C: number of outcomes among control animals; [e]Vag excret or uterus: vaginal excretion or uterus; [f]Total mat bct: total maternal bacteriology; [g]These trials used animals that were not in their first pregnancy; [h]UN: uninformed; [i]SC: subcutaneous; [j]ICD: intracaudal; [k]These trials challenge non-pregnant animals; [l]ID: intradermal; [m]m: months; [n]In this trial, 8 animals were vaccinated during early pregnancy. The vaccine and challenge doses are in CFU (colony forming unit).

Supplementary Table S9 – Detailed results on the meta-analysis for comparisons among the subgroups for abortion and infection.

Meta-analysis subgroup	N Trials	RR[b] (95% CI[c])	seRR[a]	P value	Q-test[d]	[e]I^2 (95% CI)
Abortion						
S19 10^8	3	0.40 (0.21 – 0.75)	1.38	< 0.05	4.72	0.58 (0.00 – 0.88)
S19 10^9	3	0.25 (0.12 – 0.52)	1.45	< 0.001	3.35	0.40 (0.00 – 0.82)
S19 10^{10}	5	0.53 (0.40 – 0.71)	1.16	< 0.001	5.48	0.27 (0.00 – 0.71)
RB51 10^{10}	4	0.31 (0.16 – 0.61)	1.41	< 0.001	2.23	0.00 (0.00 – 0.79)
Infection						
S19 10^8	4	0.70 (0.37 – 0.99)	1.29	< 0.05	9.22	0.67 (0.05 – 0.89)
S19 10^9	4	0.28 (0.18 – 0.42)	1.24	< 0.001	1.65	0.00 (0.00 – 0.72)
S19 10^{10}	7	0.59 (0.38 – 0.94)	1.26	< 0.05	20.89	0.71 (0.38 – 0.87)
RB51 10^{10}	5	0.43 (0.27 – 0.59)	1.27	< 0.001	4.77	0.16 (0.00 – 0.83)
[f]Non_Preg S19 10^{10}	3	0.38 (0.23 – 0.61)	1.28	< 0.001	1.56	0.00 (0.00 – 0.87)

[a]seRR: Risk ratio error; [b]RR: Risk ratio; [c]CI: confidence interval; [e]Q-test: Cochrane's Q-statistic; [f]I^2: Higgin's & Thompson's I2; [f] Non_Preg S19 10^{10}: animals challenged non-pregants.

5 Final Conclusion

In summary, the results of this thesis suggest that the doses of bovine brucellosis vaccines recommended by the OIE should be revised, whereas the most effective dose, according to this meta-analysis, is 10^9 CFU for S19 and 10^{10} CFU for RB51, both by subcutaneous route, at a single dose. This way of administering vaccines proved to be the most suitable for the prevention of abortion *lato sensu* and infection by brucellosis in cattle. For S19 vaccine, with the suggested dose, it would be possible to commercialize 50-80 times more doses for the same amount of CFU produced. This reduction in countries where vaccine production is difficult represents a huge gain for bovine brucellosis control and prevention programs. However, the results found here allow other studies to be designed such as a meta-analysis on vaccine effectiveness in the field, so that the "phase 3" and "phase 4" of vaccine studies can also be reviewed and recalculated, with the intention of reiterating or disagreeing with the results found with experimental studies.