UNIVERSIDADE FEDERAL DE LAVRAS

CARINE RODRIGUES PEREIRA

OCCUPATIONAL BRUCELLOSIS AMONG VETERINARIANS IN MINAS GERAIS STATE, BRAZIL

LAVRAS – MG
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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Ciências Veterinárias, área de concentração em Sanidade Animal e Saúde Coletiva, para obtenção do título de Mestre.

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I dedicate to my parents Geraldo and Valéria for their unconditional love, support and encouragement.

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ABSTRACT

Brucellosis is a zoonotic disease of remarkable importance worldwide, with a strong occupational chacter that affects mainly farmers, abattoir workers, microbiologists, hunters and veterinarians. The focus of this dissertation was to conduct a systematic review and meta-analysis about occupational exposure to *Brucella* and identify the main risks factors in each group exposed to the pathogen, as well as to determine the prevalence and risk factors of occupational brucellosis and accidental exposure to S19 and RB51 vaccine strains among veterinarians registered to perform brucellosis vaccination in cattle in Minas Gerais, Brazil. The systematic review was conducted based on PRISMA recommended guidelines. The meta-analysis was performed using three case controls studies. Data from epidemiological survey was collected by means of an online questionnaire. Three hundred and twenty nine veterinarians were included in the analyzes, using a stratified random sampling. A multivariable logistic regression analysis was used to evaluate the predictors of accidental exposure to bovine brucellosis vaccines. The main risk factors and exposure sources involved in the occupational infection were not use of personal protective equipment (PPE), direct contact with animal fluids and accidental exposure to live attenuated *Brucella* vaccines. The meta-analysis demonstrated that laboratory workers, animal breeders and abattoir workers had 3.47 [95% confidence interval (CI); 1.47 - 8.18] times more chance to become infected by *Brucella* than other professionals that have no contact with the possible sources of infection. In the cross sectional study, it was identified that 32.83% (108/329) [95% confidence interval (CI): 27.78 to 38.19%] of the veterinarians reported having been accidentally exposed to S19 or RB51 strains. The risk associated with this outcome included score of PPE use during work [odds ratio (OR), 0.94; 95% CI: 0.89 to 0.98] and score of knowledge about brucellosis symptoms, classified in mean (OR, 0.26; 95% CI: 0.07 to 0.87) or good (OR, 0.22; 95% CI: 0.07 a 0.62) compared to poor knowlegde. In addition, 4.56% (15/329) (95% CI: 2.57 to 7.41%) of veterinarians self reported brucellosis, of which 46.67% (7/15) considered that the disease was due to accidental exposure to S19 or RB51 strains. Hence, it was concluded that the lack of knowledge about brucellosis among exposed professionals, added to some behaviors such as negligence in the use of PPE, increases the probability of infection with *Brucella* spp.

Key words: *Brucella*. Vaccine accident. Occupational disease.

RESUMO

A brucelose é uma doença zoonótica de grande importância em todo o mundo, com um forte caráter ocupacional que afeta principalmente fazendeiros, trabalhadores do matadouro, microbiologistas, caçadores e veterinários. O foco desta dissertação foi realizar uma revisão sistemática e meta-análise sobre a exposição ocupacional a bactérias do gênero *Brucella* e identificar os principais riscos de infecção em cada grupo exposto ao patógeno, bem como determinar a prevalência e os fatores de risco da brucelose ocupacional e exposição acidental às amostras vacinais B19 e RB51 entre os veterinários registrados para realizar a vacinação contra brucelose em bovinos em Minas Gerais, Brasil. A revisão sistemática foi realizada com base nas diretrizes recomendadas pelo PRISMA. A meta-análise foi realizada usando três estudos de caso controle. Os dados do levantamento epidemiológico foram coletados por meio de um questionário online. Trezentos e vinte e nove veterinários foram incluídos nas análises, usando uma amostragem aleatória estratificada. Uma análise de regressão logística multivariada foi utilizada para avaliar os preditores de exposição acidental a vacinas contra a brucelose bovina. Os principais fatores de risco e fontes de exposição envolvidos na infecção ocupacional foram o não uso de equipamentos de proteção individual (EPI), contato direto com fluidos animais e exposição acidental a vacinas vivas de *Brucella* atenuadas. A metanálise demonstrou que trabalhadores de laboratório, criadores de animais e trabalhadores de matadouros tiveram 3,47 [intervalo de confiança de 95% (IC); 1,47 - 8,18] vezes mais chance de se infectar por *Brucella* do que outras profissões que não têm contato com as possíveis fontes de infecção. Foi identificado no estudo transversal que 32,83% [intervalo de confiança (IC) de 95%: 27,88 a 38,19%] dos veterinários relataram ter sido acidentalmente expostos a B19 ou RB51. O risco associado a esse desfecho incluiu a pontuação do uso de EPI durante o trabalho [odds ratio (OR), 0,94; IC95%: 0,89 a 0,98] e escore de conhecimento sobre sintomas de brucelose, classificados em médio (OR, 0,26; IC95%: 0,07 a 0,87) ou bom (OR, 0,22; IC95%: 0,07 a 0,62) comparados ao conhecimento ruim. Além disso, 4,56% (15/329) (IC 95%: 2,57 a 7,41%) dos veterinários autorelataram brucelose, dos quais 46,67% (7/15) consideraram que a doença foi devido à exposição acidental a B19 ou RB51. Conclui-se que a falta de conhecimento sobre a brucelose entre profissionais expostos, somada a alguns comportamentos como a negligência no uso de EPI, aumenta a probabilidade de infecção por *Brucella* spp.

Palavras-chave: *Brucella*. Acidente vacinal. Doença ocupacional

SUMMARY

1. GENERAL INTRODUCTION

Brucellosis is a disease of great importance in public health around the world, affecting especially individuals that have direct contact with sick animals and their contaminated secretions, as well as positive *Brucella* spp. cultures and live attenuated anti-*Brucella* vaccines (S19, RB51 and REV-1). The difficulty of diagnosis, the severity and chronicity of the clinical signs and the long period of antibiotic therapy recommended in the treatment, reinforce the great impact of the human brucellosis in infected individuals. Currently, it is imperative to have a global and undissociated view of how the different occupational groups interact with the animals and the environment in their surroundings, through a One Health perspective.

Therefore, in order to improve knowledge about brucellosis as an occupational disease and to have a broad view of the literature, the first aim of this dissertation was to conduct a systematic review and meta-analysis on the main risk factors to *Brucella* spp. infection in the groups most affected by the disease as a result of their work activities worldwide. In addition to this global view, an observational study was carried out at state level to determine the prevalence of accidental exposure to anti- *Brucella abortus* vaccines and occupational brucellosis, as well as to identify the risk factors associated with both outcomes among veterinarians registered to perform brucellosis vaccination in Minas Gerais state, Brazil.

CHAPTER 1: Formatted according to the submission guidelines of Plos Neglected Tropical Diseases

Occupational exposure to human brucellosis infection: a systematic review and meta-analysis

Short title: Occupational brucellosis exposure

Abstract

Background

Brucellosis is a neglected zoonotic disease of remarkable importance worldwide. The focus of this systematic review was to investigate occupational exposure to bacteria of genus *Brucella* and identify the main infection risks in each group exposed to the pathogen.

Methodology/Principal Findings

Seven databases were used to identify papers related to occupational brucellosis: CABI, Cochrane, Pubmed, Scielo, Science Direct, Scopus and Web of Science. The search resulted in 6123 studies, of which 62 were selected using the quality assessment tools guided from National Institutes of Health (NIH) and Case Report Guidelines (CARE). Five different job-related groups were considered greatly exposed to the disease: rural workers, abattoir workers, veterinarians and veterinary assistants, laboratory workers and hunters. The main risk factors and exposure sources involved in the occupational infection were direct contact with animal fluids, not use of personal protective equipment, accidental exposure to live attenuated *Brucella* vaccines and non-compliance with biosafety standards. *Brucella* species frequently isolated from job-related infection were *Brucella melitensis*, *Brucella abortus*, *Brucella suis* and *Brucella canis*. In addition, a meta-analysis was performed using the case-controls studies and demonstrated that animal breeders, laboratory workers and abattoir workers have 3.47 [95% confidence interval (CI); 1.47 - 8.18] times more chance to become infected with brucellosis than other professions that have no contact with the possible sources of infection.

Conclusions

This systematic review improved the understanding on the epidemiology of brucellosis as an occupational disease. Rural workers, abattoir workers, veterinarians, laboratory workers and hunters were the groups more exposed to *Brucella* spp. infection. Moreover, it was observed that the lack of

knowledge about brucellosis among frequently exposed professionals, added to some behaviors, negligence in the use of individual and collective protection measures, increasing the probability of infection.

Author summary

Brucellosis is a bacterial infection of major importance worldwide, that affects a great diversity of species, not only domestic and wild animals, but also humans. Due to its form of transmission, direct or indirect contact with infected animals or their contaminated biological products, the disease exhibits a strong occupational character. This systematic review addressed the main occupations affected by *Brucella* infection, due to the regular exposure to aerosol and contact of non-intact skin (e.g. wound and abrasion) with infected materials, such as carcasses, viscera and live attenuated anti-*Brucella* vaccines. The main risk factors for the disease were identified, as well as the most common forms of exposure to the pathogen. In addition, the most frequently *Brucella* species isolated from farmers, abattoir workers, veterinarians and veterinary technicians, laboratory workers and hunters were also described. The constant contact with the pathogen, the lack of information and instructions to occupational groups exposed, as well as the low adhesion to personal protective equipment in the work environment are determining factors for the occurrence of brucellosis among these individuals.

Introduction

Brucellosis is one of the most common anthropozoonosis in the world, with approximately 500,000 new human cases reported annually to the World Health Organization (WHO) (1). Accidental exposure of humans through the ingestion of untreated dairy products, unprotected contact with infected animals or contaminated biological materials, and accidental exposure to anti-*Brucella* vaccines used in veterinary practice are the major forms of disease transmission, which has a strong occupational feature (2, 3). The professionals most exposed to the pathogen are breeders and animal handlers, butchers, laboratory workers, veterinarians and veterinary assistants, and hunters (4).

The pathological condition caused by the human infection by bacteria of the genus *Brucella* is characterized by non-specific acute symptoms, such as fever, malaise, chills, weight loss and arthralgia. In some cases, brucellosis can evolve to chronic signs, which can affect a large number of systems and cause osteomyelitis, orchitis and endocarditis, among other manifestations (1, 5). Treatment of the disease is usually long, and is intended to control the acute form and prevent chronic evolution. The administration of two synergistic antibiotics, doxycycline and rifampicin or doxycycline and an aminoglycoside, is normally recommended (among other possible therapies), and the treatment should last a period of at least six weeks. The discontinuity of chemotherapy is responsible for debilitating complications and relapses (6), and may lead to permanent sequelae incapacitating the individual for work (7). On a global basis, brucellosis is one of the 20 highest-ranked conditions with impact on impoverished people (8). Damage caused by the disease in individuals' quality of life is intangible and the economic losses attributed to the infection in humans are associated to the costs of hospital treatment, drugs and absence from work due to disabling feature of the disease in its severe form (7).

The prevention of brucellosis transmission among occupations that directly deal with animals or their products relies on effective defensive measures, as the adoption of personal protective equipment (PPE) during activities involving the possibility of *Brucella* infection (9). Manipulation of potentially infected animals, biological materials and anti-*Brucella* vaccines are risk factors of remarkable importance for human brucellosis; however, the more detailed knowledge about particular risk factors to each occupation, as well as the measurement of these risks is still deficient. In fact, there is a need for more accurate data on the epidemiology of job-related brucellosis to allow the implementation of more effective preventive measures, which will reduce the impact of the disease in groups exposed by their work activities. The availability of these information could also be translated into health protection behaviors among susceptible professionals. Thus, the aims of this systematic review were (i) to identify high quality studies that reported and evaluated occupational exposure to brucellosis, (ii) to evaluate the main risk factors of each exposed group (rural workers, abattoir workers, laboratorists, veterinarians, veterinary technicians and hunters), and (iii) to estimate, by means of a meta-analysis, the odds of individuals occupationally exposed to *Brucella* spp. become infected, compared to individuals not exposed to direct animal contact or their biological fluids.

Methods

The guidelines of PRISMA statement (Preferred Reported Items for Systematic Reviews and Meta-Analyses) were formally adopted in this review and can be seen in additional file 1 (S1 appendix).

Search strategy

The literature review included original papers published between 1931 and 2018. The search was conducted on May 16, 2018. All the 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. An overview of the search terms is shown in supplementar file 2 (S2 Appendix).

Studies selection

Titles of each one of the papers identified during the initial search were first selected by the first author. In the second stage, for those studies selected based on their titles, two reviewers independently evaluated each abstract. Subsequently, full text of the selected papers based on the abstracts were evaluated by two reviewers in terms of its relevance and by means of inclusion/exclusion criteria. When these reviewers disagreed over the inclusion or exclusion of a paper, a third reviewer was responsible for the final decision. Further, the reference lists of selected papers were reviewed in order to find pertinent studies not identified during the initial search.

Inclusion and exclusion criteria

The following characteristics were considered for the inclusion of articles: (i) approach on *Brucella* spp. and (ii) concerning occupational exposure to brucellosis infection or to *Brucella* spp. Articles focusing on (i) animal brucellosis, (ii) genetics, immunology, microbiology or drug therapy, and (iii) written in languages other than English, Spanish, French and Portuguese were excluded. Full inclusion and exclusion criteria are shown in additional file 3 (S3 Appendix).

Type of studies

Original and full text papers, using quantitative or qualitative data, as cohort, case control, cross sectional, case series and case reports were included. Reviews were excluded.

Data extraction and quality assessment

Data were extracted from papers by one of the reviewers and were subsequently checked for accuracy by the other reviewer. Disagreements regarding data extraction among reviewers were solved by consensus. Extracted data included: first author, geographic location, study period, participants, positive individuals, study design, diagnostic method and cut off values, *Brucella* species isolated, identification of occupational exposure, predictors of transmission, potential risks factors for the development of brucellosis among high-risk groups and possible molecular confirmations from the source of infection. The case definitions described in each study by the respective author(s) were considered. The quality of cohort, case control, cross sectional and case series studies was evaluated using the quality assessment tools from the National Heart, Lung and Blood Institute (NHLBI), and CARE (Case Report) checklist was used for quality assessment of case reports (10).

Meta-analysis

Case control studies were selected to estimate the odds of individuals occupationally exposed to *Brucella* spp. become infected, compared to individuals without occupational risk; and the homogeneity among the studies was verified using Cochrane's Q test. The total variability related to among-study variations was reflected in the Tau ^ 2, which was estimated by the DerSimoninan-Laird method. The pooled odds ratio (OR) of the studies was obtained through a random effect modeling and by the adoption of the Mantel-Haenszel estimator. The meta-analysis was performed with R statistical software 3.5.2 (11), using the meta package (12).

Results

The search strategy adopted identified a total of 6123 papers; 454 duplicates were excluded, and 238 full texts were assessed for eligibility. Subsequently, 62 papers were included in quality level assessment and data synthesis appraisal, after a thorough review (S4 Appendix). The background characteristics (country, period, participants, positive individuals, study design, diagnostic, reported predictors of brucellosis transmission to human and molecular links) identified in these articles are shown in additional file 5 (S5 Appendix).

The assessment of geographical origin on selected job-related brucellosis papers showed that seven studies were from Africa, seventeen from America, twenty-two from Asia and sixteen from Europe (Fig 1A). Regarding to the year of publication, except for the 1970s, the number of studies published about human brucellosis with occupational feature increased every decade (Fig 1B). Indirect methods, as agglutination tests, indirect-ELISA, 2-mercaptoethanol, complement fixation, among others, were the main tests used to human brucellosis diagnosis in the studies, which observed 1432 individuals occupationally infected. Moreover, the use of direct methods for the diagnosis, such as isolation and polymerase chain reaction (PCR), also revealed 106 positive individuals, being identified *Brucella melitensis* (n = 71), *Brucella suis* (n = 24), *Brucella abortus* (n = 10) and *Brucella canis* (n = 1). The Fig 2 shows the *Brucella* species most frequently identified (a) and the distribution of brucellosis cases by country (b) according to occupational group affected.

Fig 1. Geographical and temporal distribution of selected studies. (a) Distribution and frequency of the selected studies on occupational brucellosis according to the country. (b) Distribution and frequency of the selected studies on occupational brucellosis according to continent and year of publication, from 1960 and 2018.

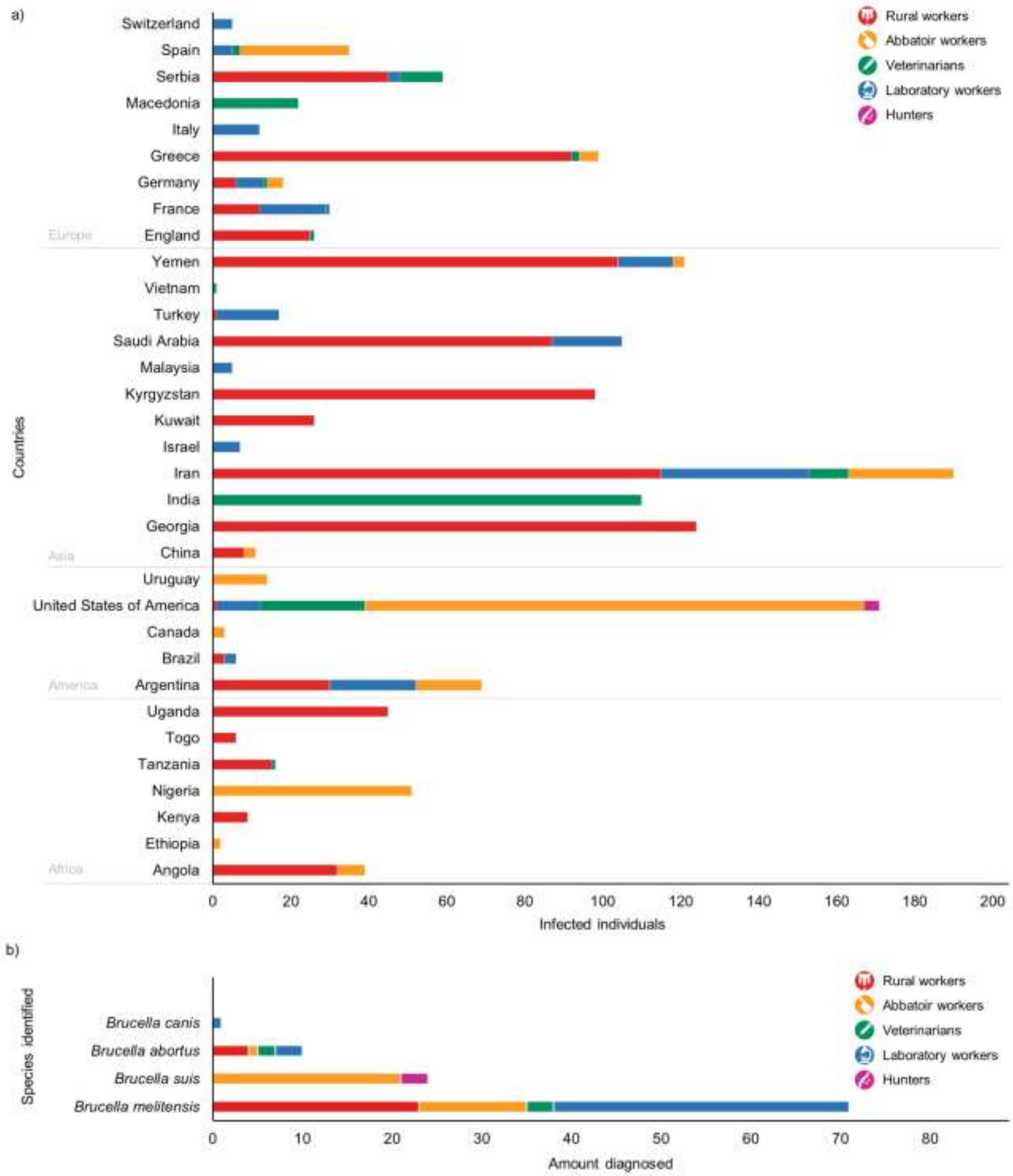

Fig 2. Distribution of occupations affected by brucellosis, as a result of their work activities, according to the country (a) and species of *Brucella* identified (b) (direct diagnostic methods).

Rural workers

Farmers, shepherds and livestock breeders were the leading groups affected by brucellosis, with 870 positive individuals described in twenty-four studies (2, 13-35), of which the most part was carried out in Asia (n = 549), Europe (n = 180), Africa (n = 107) and the minority in America (n = 34). Direct contact with potentially infected cattle, goats and sheep during labor activities, such as calving, barn cleaning and herd vaccination, were described in the studies as potential sources of infection of *Brucella* spp. to rural workers (Table 1). Irrefutable evidence of animal-to-human brucellosis transmission was observed by a study conducted in Argentina, in which the same genotype of *B. melitensis* was observed in milk (n = 17) and colostrum (n = 11) samples from goats and in rural workers (n = 14) who lived near the animals (33). Moreover, another study also identified that aborted fetus remains were abandoned in the pasture and eventually ingested by dogs and pigs, in some properties in Angola (28).

Table 1: Farm animal species related to brucellosis occupational transmission among infected rural workers.

Study	Country	Total of workers	Contact	
			Cattle	Small ruminants
(2)	USA	1	1	0
(17)	Uganda	19	0	19
(21)	Brazil	2	2	0
(26)	France	11	11	NR
(28)	Angola	32	32	NR
(31)	England	1	1	0
(33)	Argentina	32	0	32
(34)	England	1	1	0
	Total	99 (100.00%)	48 (48.48%)	51 (51.52%)

NR = not reported; USA = United States of America

Abattoir workers

A total of 292 individuals working in slaughterhouses were described brucellosis-positive in fourteen articles (14, 15, 18, 28, 29, 36-44). Most of these individuals were from America (n = 162), Africa (n = 60), Europe (n = 37) and the minority from Asia (n = 33). The main type of pathogen exposure reported was contact with animal fluid, such as aborted fetus, placenta and viscera. Accidental contact with these fluids was described in three studies: in Spain and Ethiopia, 12.26% (13/106) and 48.72% (76/156) of slaughterhouse workers, respectively, reported cutting themselves with dirty sharp blades

(40, 41), and in China, 100.00% (3/3) of pharmaceutical employees, who worked processing sheep placenta, reported having already splashed animal fluids on their faces (42). This situation aroused the interest of several authors to understand which PPE were used by this group of professionals (Table 2).

Table 2: Use of personal protective equipment (PPE) among slaughterhouse workers occupationally infected by *Brucella* spp.

Study	Country	Total of workers	PPE not used				
			Gloves	Masks	Goggles	Boots	Apron
(36)	Nigeria	54	2	NR	NR	NR	NR
(38)	Iran	198	25	82	20	113	101
(39)	Uruguay	14	NR	NR	0	NR	NR
(40)	Spain	28	19	18	16	NR	NR
(41)	Ethiopia	156	29	NR	NR	NR	NR
(42)	China	3	3	3	NR	NR	NR
(43)	Argentina	17	0	0	0	NR	NR
	Total	470	78/456 (17.11%)	103/246 (41.87%)	36/257 (14.01%)	113/198 (57.07%)	101/198 (51.01%)

NR = not reported; the percentage was calculated based on the total individuals interviewed about PPE

Veterinarians and veterinary assistants

Veterinarians and veterinary assistants showed to be largely exposed to *Brucella* spp., totalizing 189 individuals with positive diagnostic of brucellosis. These infections probably related to their occupational activities were reported by fifteen articles (2, 14, 18, 19, 23, 26, 30, 38, 45-51), mostly from Asia (n = 121), Europe (n = 40), America (n = 27) and the minority from Africa (n = 1). Manipulation of anti- *Brucella* live attenuated vaccines was the most reported exposure source, described in seven studies (Table 3). Of these, three were able to establish an epidemiological link between the vaccine strain and the strain responsible for the infection in the veterinarians: *B. abortus* strain RB51 was isolated from a surgical wound three days after a self-inoculation (2); *B. abortus* strain 19 was cultured from a discharge, from the injection site, obtained on the eight day after a needlestick injury (49); and *B. melitensis* strain REV-1 was isolated from blood culture of two veterinarians, several months after the accidental exposure (46). In addition to this type of exposure, veterinarians and veterinary assistants also reported to perform other activities associated with a high risk of infection, such as attending parturitions and infertility cases, and handling aborted fetus, retained placenta and stillbirths (18, 19, 26, 45). Furthermore, the use of PPE in some cases was considered inadequate (38, 51).

Table 3: Adverse events or occupational brucellosis in veterinarians and veterinary assistants associated with accidental exposure to anti- *Brucella* live attenuated vaccines.

Study	Country	Total of workers	Vaccine strain		
			RB51	S19	REV-1
(2)	USA	19	19	0	0
(13)	Georgia	1	NR	NR	NR
(18)	Greece	41	0	0	41
(46)	Spain	2	0	0	2
(49)	USA	1	0	1	0
(50)	USA	1	0	1	0
(52)	India	5	0	5	0
	Total	70	19 (27.14%)	7 (10.00%)	43 (61.43%)

USA = United States of America; NR = not reported

Laboratory workers

Brucellosis related to laboratory practices was largely reported: 24 papers described this transmission in 183 individuals, of which the majority was from Asia (n = 98), Europe (n = 49) and the minority from America (n = 36) (14, 15, 23, 26, 53-72). The main factors possibly related with the infection were working outside a safety cabinet, being at the laboratory during or after an accident, failure suspecting brucellosis as a possible diagnosis and sniffing culture plates (Table 4). Two papers reported infection in individuals working outside a laboratory facility, but in indirectly related departments with the presence of *Brucella* positive cultures within the environment. The first case was in a S19 manufacturing plant, where 21 workers were infected probably by the vaccine strain, in Argentina (70); whereas, the second occurred in a waste treatment plant, where an employee stuck his foot in a needle contaminated with the *B. suis* biovar 1 reference strain 1330 (55) identified by molecular genotyping methods. The epidemiological link (biotyping) between the source of accidental exposure and the patient's isolate was also established in other reports of brucellosis among laboratory technicians in Switzerland (*B. melitensis* biovar 3) and Italy (*B. abortus* biovar 1) (58, 59). Moreover, the same biovar was also identified in 8 laboratory workers, during an brucellosis outbreak in the United States of America (69). Additionally, in France, the occupational brucellosis represented from 2004 to 2013 46% of domestic cases (all laboratory exposure) and for 94.1% of the brucellosis-positive patients the respective paired strain was identified at molecular level (26).

Table 4: Types of exposition associated with occupational transmission of brucellosis reported by infected laboratory workers.

Study	Country	Total of workers	Possible cause of infection			
			Outside safety cabinet	Accident reported	Wrong diagnostic*	Sniffed plates
(53)	Saudi Arabia	4	2	2	0	0
(55)	Spain	1	0	1	0	0
(56)	Turkey	3	0	0	0	3
(58)	Italy	12	0	12	0	0
(59)	Switzerland	2	0	0	2	0
(60)	Malaysia	1	0	0	1	0
(61)	Saudi Arabia	2	1	0	0	1
(62)	Spain	4	4	0	0	0
(63)	Saudi Arabia	1	0	0	0	1
(64)	USA	2	2	0	0	0
(66)	Brazil	1	0	1	0	0
(68)	USA	1	1	0	0	0
(70)	Argentina	5	0	5	0	0
(71)	Argentina	1	1	0	0	0
	Total	40	11 (27.50%)	21 (52.50%)	3 (7.50%)	5 (12.50%)

USA = United States of America; * = Brucellosis not included as possible diagnosis by the clinician

Hunters

Job-related exposure was described in feral swine hunters in two papers, totalizing 4 infected individuals, all from American continent (73, 74). Contact with animal fluid was reported, although 50% of the individuals mentioned the adoption of personal protective measures during the handling of feral swine. Furthermore, a frozen sausage and a tenderloin, from a feral swine hunted by two men, were positive for *B. suis* isolation, and had multiple-locus variable-number of tandem repeats analysis (MLVA) signatures identical to a *B. suis* strain isolated from one of the patients (73).

Meta-analysis

Individuals who perform risky labor activities, such as farm laboring, or employees from slaughterhouses and laboratories showed 3.47 [95% confidence interval (CI); 1.47 - 8.18] times more chance to become infected with *Brucella* strains than people who develop other occupational activities (Fig 3).

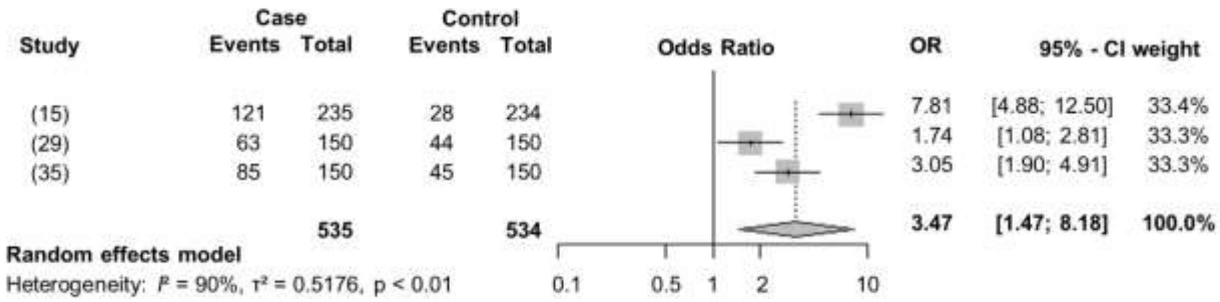

Fig 3: Forest plot for brucellosis infection risk among professions exposed and not exposed to *Brucella* spp. during they occupational activities.

Discussion

Brucellosis is a worldwide widespread disease of great importance to public health and has a strong occupational character, with certain professions being more commonly affected by the disease (4). Therefore, the efforts of this systematic review and meta-analysis were focused on detailing the understanding of the main risk factors associated with occupational brucellosis among occupations considered to be more exposed to the agents. Our findings showed, in fact, a greater chance of infection among field occupations that have direct contact with animals and their products, as well as indicated the main situations of risk and behaviors associated with infection for each evaluated profession. Information provided by this study is essential to design strategies to minimize the occurrence of occupational brucellosis and to guide specific health protection behaviors to people occupationally exposed.

Although brucellosis is a widespread zoonotic disease, no high-quality studies concerning to occupational cases from Oceania were selected, which could be explained by the low occurrence of the disease in animals in the region. Likewise, the differences in the number and emergence of publications among the continents may be due to divergences in the structure of brucellosis surveillance systems and in the epidemiological situation of the diseases in animals (Fig 1B), since animal brucellosis precede and are closely associated with human brucellosis, especially occupational (75). Moreover, the increased amount of publications from the 80's could be associated with the growing importance of the disease in humans and the development of new diagnostic techniques. In fact, the oldest publications selected were from countries that have implemented their brucellosis control and prevention programs in the 10s, 20s and 30s, such as the United States and Canada, in America, and Great Britain, in Western Europe (76-78). On the other hand, some countries in Asia, Latin America and Africa, although endemic for animal brucellosis, have not yet reached satisfactory levels of disease control and often report insufficient data on the true prevalence of the disease. Additionally, in these regions poor interaction between human and veterinary medicine are generally observed (7, 79), which could explain the later appearance of scientific publications in the area among the selected papers. However, it is very important to mention that the number of infected individuals and the number of papers published by country do not have a direct relationship with the actual prevalence of occupational brucellosis in that locality, but is more related to scientific interests of local researchers.

The indirect methods were mostly common used for the diagnosis of brucellosis, which could be attributed to the lower cost of serologic tests compared to PCR and microorganism isolation, as well as to the safety issues and time saving process compared to bacterial culture (80-82). Even though not widely used, direct methods have the great advantage of being able to identify the *Brucella* species responsible for the infection, supporting a better understanding of the etiopathogenesis of the disease among the different occupational groups included in this study.

Rural workers are among the group most affected by brucellosis, mainly caused by *B. abortus* (83), however, the infection caused by this species is frequently subclinical, with nonspecific clinical signs (84), having as a consequence the underdiagnosis. This may explain the lower number of rural workers infected by this species, compared to *B. melitensis* observed in the present study (Fig 2A). These results are especially important to public health, since *B. melitensis* is the most pathogenic species of *Brucella* spp. for humans, and the disease may progress to the development of debilitating symptoms, with severe involvement of multiple organs and systems, and high cost of hospitalization due to the prolonged therapy recommended (85). The close contact of rural workers with small ruminants, preferred hosts of this species, was identified as the main form of acquisition of the disease among these individuals (Table 1), which has been confirmed by the identification of a high genetic similarity between *B. melitensis* strains isolated from occupationally infected workers and from goat milk samples.

The second group most affected by occupational brucellosis, mainly by *B. suis*, followed by *B. melitensis* and *B. abortus* (Fig 2A) were butchers and abattoir workers, probably due to the regular manipulation of sharp objects and to close contact with potentially infected animals and their indoors organs. Airborne and conjunctival routes were considered important to the transmission of brucellosis among this group (86), especially in closed places, such as slaughterhouses, in which direct contact with contaminated viscera and secretions occurs. The hazard was increased when prophylactic measures were not properly adopted, as highlighted by the low adherence of PPE use, such as gloves, masks, googles, boots and apron (Table 2), leading to unhealthy working conditions. In addition, the low educational level of abattoir workers, as well as insufficient knowledge about brucellosis, particularly on its transmission and clinical signs, increases the risk of these professionals becoming infected and reinforce the importance of implementing educational measures to advise about the need to use PPE (36, 41, 42).

Subsequently, veterinarians and veterinary assistants comprised the third occupational group most affected by brucellosis. In addition to contact with secretions and excretions of potentially infected animals, activities inherent to their work (51), many of these professionals were also exposed to *Brucella* live attenuated vaccines (REV-1, S19 and RB51) (Table 3), which are an important source of the infection for humans (2). Accidental exposures to brucellosis live attenuated vaccines are especially severe when they occur with RB51, since antibodies against this strain are not detected by routine serological tests and RB51 is resistant to rifampicin, one of the preferential drugs to treat human brucellosis (87). In fact, the accidental exposure to brucellosis vaccines has great significance to brucellosis cases among veterinarians and assistants, being confirmed by direct diagnostic methods that revealed *Brucella* infection caused by *B. melitensis* and *B. abortus* vaccine strains (2, 46, 49). These findings strengthen the importance of use PPE not only in the care of animals, but also during the vaccination procedures.

Laboratory workers represent the fourth group most affected by the *Brucella* infection due to their labor activities. Interestingly, this group showed the greatest diversity of species isolated: *B. abortus*, *B. canis* and *B. melitensis* (Fig 2A), which could be explained by the wide variety of clinical specimens that are often handled by these professionals in the diagnostic routine. Moreover, it must be considered that this group had the largest number of *Brucella* strains isolated and identified among the occupations evaluated, probably due to greater access to direct methods of diagnosis in the environments where they were occupationally exposed. Nonetheless, albeit generally well instructed about the risk of contracting a zoonotic infection during labor activities, many laboratory workers adopted attitudes that put their own health and of their colleagues at risk, as work outside safety cabinet and sniff the plates (Table 4). *Brucella* cultures must be handled only in laboratories with biosafety level 3 or higher (88); however, due the lack of specificity of the clinical signs caused by the disease, associated with the effectiveness of public policies in some European countries (14, 26), where brucellosis occurs primarily among travelers, many physicians rarely raise the hypothesis of brucellosis when sending biological samples for laboratory analysis, leading exposure to the agent during manipulation of the clinical material by the microbiologist (89). Furthermore, accidents, as damages in the biological safety cabinet or the centrifuge, may also occur in the biosafety level 3 laboratory, reinforcing that training activities to the staff must be periodically carried out in order to ensure cautious manipulation of positive *Brucella* cultures, as well as regular laboratorial equipment maintenance (89). Indeed, adherence to rigorous infection control measures are important from the receipt to the proper disposal of biological materials, since in this occupational group not only microbiologists but also people working in the laboratory waste processing were affected (55).

The occupation with the lowest number of infected individuals identified was the group of hunters, which differently from the previous groups exhibited exclusively *B. suis* isolates (Fig 2A). The primary route of transmission for *B. suis* in general population is usually through the consumption of contaminated meat, unlike *B. abortus* and *B. melitensis*, which have milk as their major source of infection (5, 86). The presence of bacteria in the muscular tissues of boars is sufficient to cause infection in humans, which makes hunting and evisceration even more risky, especially when carried out without the proper use of individual protection measures.

The occupational character of human brucellosis is supported by the results generated from the meta-analysis of 4 case-control studies, which showed that animal breeders, laboratory workers and abattoir workers exhibited significant more likely to become infected with *Brucella* strains than people who develop other job-related activities (OR 3.47; 95% CI: 1.47 to 8.18) (Fig 3). The low number of selected studies with a case control design (n = 3) and the great heterogeneity observed among the articles resulted in the small number of high-quality papers eligible for meta-analysis. However, it is important to take into account that despite the low number of studies used in the meta-analysis, the total number of individuals analyzed (n = 1069) and those with occupational brucellosis (n = 269) was expressive, reflecting in the robust results observed (Fig 3). These data revealed the weight of exposure during labor activities for the occurrence of human brucellosis, being essential to the design of strategies to minimize its occurrence.

The greatest strengths of this paper are that it is based on the PRISMA statement, the search was performed in seven scientifically validated databases and the quality assessment of papers were through NIH and CARE guidelines, which allowed the accomplishment of a metanalysis and mitigated possible bias among studies. On the other hand, there are some limitations such as the differences among case definitions and diagnostic capacity of different studies, especially due to the diversity of diagnostic techniques employed (see detailed information on supplementar file 5 (S5 Appendix)).

Furthermore, some papers were not available despite all efforts through the university databases, e-mails, scientific social midia and commute request.

This systematic review provided a meticulous understanding on the risk factors of each of the leading occupations (farmers, abattoir workers, veterinarians, laboratorists and hunters) closely related with *Brucella* infection. Furthermore, our results also revealed the great lack of information from these occupational groups about the importance of applying preventive measures to minimize the risk of transmission of brucellosis while working. This data can be used as one step towards in adopting a One Health approach to brucellosis control, which could be conducted more efficiently and strategically, in order to reduce the incidence of the disease not only in humans, but also in animals and in the environment.

In conclusion, our results reinforced the strong occupational character of human brucellosis, especially among rural workers, slaughterers, veterinarians and veterinary assistants, laboratory workers and hunters, and revealed the specific risks associated with each occupation. Moreover, it was observed that the lack of knowledge about brucellosis among frequently exposed professionals, added to some behaviors, such as negligence in the use of individual and collective protection measures, increased the probability of infection.

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References

1. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. The Lancet Infectious Diseases. 2006;6(2):91-9.

2. Ashford DA, di Pietra J, Lingappa J, Woods C, Noll H, Neville B, et al. Adverse events in humans associated with accidental exposure to the livestock brucellosis vaccine RB51. Vaccine. 2004;22(25-26):3435-9.

3. Brough HA, Solomon AW, Wall RA, Isaza F, Pasvol G. Brucellosis acquired by eating imported cheese. Journal of paediatrics and child health. 2011;47(11):840-1.

4. Corbel MJ, Elberg SS, Cosivi O. Brucellosis in humans and animals. World Health Organization. 2006.

5. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. The New England Journal of Medicine. 2005;352(22):2325-36.

6. Godfroid J, Scholz HC, Barbier T, Nicolas C, Wattiau P, Fretin D, et al. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Preventive Veterinary Medicine. 2011;102(2):118-31.

7. McDermott J, Grace D, Zinsstag J. Economics of brucellosis impact and control in low-income countries. Scientific and Technical Review of the Office International des Epizooties. 2013;32(1):249-61.

8. Perry B, Randolph T, McDermott JJ, Sones KR, Thornton P. Investing in animal health research to alleviate poverty. International Livestock Research Institute. 2002.

9. Dias EC, Almeida IM. Doenças relacionadas ao trabalho. Ministério da Saúde. 2001.

10. Gagnier JJ, Riley D, Altman DG, Moher D, Sox H, Kienle G. The CARE guidelines: consensus-based clinical case reporting guideline development. Deutsches Arzteblatt international. 2013;110(37):603-8.

11. Team RDC. R: A language and environment for statistical computing. 3.5.2 ed. Vienna, Austria: R Foundation for Statistical Computing. 2018.

12. Schwarzer G. meta: meta-analysis with R. R package version 4.9-4 ed 2019.

13. Akhvlediani T, Clark DV, Chubabria G, Zenaishvili O, Hepburn MJ. The changing pattern of human brucellosis: clinical manifestations, epidemiology, and treatment outcomes over three decades in Georgia. BMC Infectious Diseases. 2010;10:346.

14. Al Dahouk S, Neubauer H, Hensel A, Schöneberg I, Nöckler K, Alpers K, et al. Changing epidemiology of human brucellosis, Germany, 1962-2005. Emerging Infectious Diseases. 2007;13(12):1895-900.

15. Al-Shamahy HA, Whitty CJ, Wright SG. Risk factors for human brucellosis in Yemen: a case control study. Epidemiology and Infection. 2000;125(2):309-13.

16. Ari MD, Guracha A, Fadeel MA, Njuguna C, Njenga MK, Kalani R, et al. Challenges of establishing the correct diagnosis of outbreaks of acute febrile illnesses in Africa: the case of a likely *Brucella* outbreak among nomadic pastoralists, northeast Kenya, March-July 2005. The American Journal of Tropical Medicine and Hygiene. 2011;85(5):909-12.

17. Asiimwe BB, Kansiime C, Rwego IB. Risk factors for human brucellosis in agro-pastoralist communities of south western Uganda: a case-control study. BMC Research Notes. 2015;8.

18. Avdikou I, Maipa V, Alamanos Y. Epidemiology of human brucellosis in a defined area of Northwestern Greece. Epidemiology and Infection. 2005;133(5):905-10.

19. Cash-Goldwasser S, Maze MJ, Rubach MP, Biggs HM, Stoddard RA, Sharples KJ, et al. Risk factors for human brucellosis in northern Tanzania. The American Journal of Tropical Medicine and Hygiene. 2018;98(2):598-606.

20. Dean AS, Bonfoh B, Kulo AE, Boukaya GA, Amidou M, Hattendorf J, et al. Epidemiology of brucellosis and Q fever in linked human and animal populations in northern Togo. Plos One. 2013;8(8).

21. Gonçalves DD, Benitez A, Lopes-Mori FMR, Alves LA, Freire RL, Navarro IT, et al. Zoonoses in humans from small rural properties in Jataizinho, Parana, Brazil. Brazilian Journal of Microbiology. 2013;44(1):125-31.

22. Guney F, Gumus H, Ogmegul A, Kandemir B, Emlik D, Arslan U, et al. First case report of neurobrucellosis associated with hydrocephalus. Clinical Neurology and Neurosurgery. 2008;110(7):739-42.

23. Hasanjani Roushan MR, Mohrez M, Smailnejad Gangi SM, Soleimani Amiri MJ, Hajiahmadi M. Epidemiological features and clinical manifestations in 469 adult patients with brucellosis in Babol, Northern Iran. Epidemiology and Infection. 2004;132(6):1109-14.

24. Jia B, Zhang F, Pang P, Zhang T, Zheng R, Zhang W, et al. *Brucella* endocarditis: clinical features and treatment outcomes of 10 cases from Xinjiang, China. Journal of Infection. 2017;74(5):512-4.

25. Kozukeev TB, Ajeilat S, Maes E, Favorov M. Risk factors for brucellosis. Morbidity and Mortality Weekly Report. 2006;55(Supplement):31-4.

26. Mailles A, Garin-Bastuji B, Lavigne JP, Jay M, Sotto A, Maurin M, et al. Human brucellosis in France in the 21st century: Results from national surveillance 2004–2013. Médecine et Maladies Infectieuses. 2016;46(8):411-8.

27. Mousa AR, Elhag KM, Khogali M, Marafie AA. The nature of human brucellosis in Kuwait: study of 379 cases. Reviews of infectious diseases. 1988;10(1):211-7.

28. Mufinda FC, Boinas F, Nunes C. Prevalence and factors associated with human brucellosis in livestock professionals. Revista de Saúde Pública. 2017;51.

29. Sofian M, Aghakhani A, Velayati AA, Banifazl M, Eslamifar A, Ramezani A. Risk factors for human brucellosis in Iran: a case-control study. International Journal of Infectious Diseases. 2008;12(2):157-61.

30. Strbac M, Ristic M, Petrovic V, Savic S, Ilic S, Medic S, et al. Epidemiological characteristics of brucellosis in Vojvodina, Serbia, 2000-2014. Vojnosanitetski Pregled. 2017;74(12):1140-7.

31. Tee G. Subclinical *Brucella* infection in man. British Medical Journal. 1972;3(5823):416.

32. Thomas DR, Salmon RL, Coleman TJ, Morgan-Capner P, Sillis M, Caul EO, et al. Occupational exposure to animals and risk of zoonotic illness in a cohort of farmers, farmworkers, and their families in England. Journal of Agricultural Safety and Health. 1999;5(4):373-82.

33. Wallach JC, Samartino LE, Efron A, Baldi PC. Human infection by *Brucella melitensis*: an outbreak attributed to contact with infected goats. FEMS Immunology and Medical Microbiology. 1997;19(4):315-21.

34. Williams E. Brucellosis and the british farmer. The Lancet. 1970;295(7647):604-6.

35. Cooper CW. Risk factors in transmission of brucellosis from animals to humans in Saudi Arabia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1992;86(2):206-9.

36. Aworh MK, Okolocha E, Kwaga J, Fasina F, Lazarus D, Suleman I, et al. Human brucellosis: Seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria - 2011. Pan African Medical Journal. 2013;16.

37. Hendricks SL, Borts IH, Heeren RH, Hausler WJ, Held JR. Brucellosis outbreak in an Iowa packing house. American Journal of Public Health and the Nation's Health. 1962;52(7):1166-78.

38. Mamani M, Majzoobi MM, Keramat F, Varmaghani N, Moghimbeigi A. Seroprevalence of brucellosis in butchers, veterinarians and slaughterhouse workers in Hamadan, western Iran. Journal of Research in Health Sciences. 2018;18(1).

39. Pisani A, Vacarezza M, Tomasina F. Study of 14 cases of brucellosis in workers of a refrigerator as an occupational disease, Uruguay 2009-2010. Revista Medica del Uruguay. 2017;33(3):168-73.

40. Rodríguez Valín ME, Pousa Ortega A, Pons Sánchez C, Larrosa Montañés A, Sánchez Serrano LP, Martínez Navarro F. La brucelosis como enfermedad profesional: estudio de un brote de transmision aerea en un matadero. Revista Española de Salud Pública. 2001;75(2):159-70.

41. Tsegay A, Tuli G, Kassa T, Kebede N. Seroprevalence and risk factors of brucellosis in abattoir workers at Debre Zeit and Modjo export abattoir, central Ethiopia. Bmc Infectious Diseases. 2017;17.

42. Zhan BD, Wang SQ, Lai SM, Lu Y, Shi XG, Cao GP, et al. Outbreak of occupational brucellosis at a pharmaceutical factory in southeast China. Zoonoses and Public Health. 2017;64(6):431-7.

43. Wallach JC, García JL, Cardinali PS, Seijo AP, Benchetrit AG, Echazarreta SE, et al. High incidence of respiratory involvement in a cluster of *Brucella suis* infected workers from a pork processing plant in Argentina. Zoonoses and Public Health. 2017;64(7):550-3.

44. Bourne FM, Starkey DH, Turner LJ. Brucellosis in a Veterans' Hospital, 1963. Canadian Medical Association Journal. 1964;91(22):1139-45.

45. Arlett PR. A case of laboratory acquired brucellosis. BMJ British Medical Journal. 1996;313(7065):1130-2.

46. Blasco JM, Díaz R. *Brucella melitensis* Rev-1 vaccine as a cause of human brucellosis. The Lancet. 1993;342(8874):805.

47. Bosilkovski M, Stojanov A, Stevanovic M, Karadzovski Z, Krstevski K. Impact of measures to control brucellosis on disease characteristics in humans: experience from an endemic region in the Balkans. Infectious Diseases. 2018;50(5):340-5.

48. Campbell JI, Lan NPH, Phuong PM, Chau LB, Trung Pham D, Guzmán-Verri C, et al. Human *Brucella melitensis* infections in southern Vietnam. Clinical Microbiology and Infection. 2017;23(11):788-90.

49. Joffe B, Diamond M. Brucellosis due to self-inoculation. Annals of Internal Medicine. 1966;65(3):564-5.

50. Nicoletti P, Ring J, Boysen B, Buczek J. Illness in a veterinary student following accidental inoculation of *Brucella abortus* strain 19. Journal of American College Health. 1986;34(5):236-7.

51. Proch V, Singh BB, Schemann K, Gill JPS, Ward MP, Dhand NK. Risk factors for occupational *Brucella* infection in veterinary personnel in India. Transboundary and Emerging Diseases. 2018;65(3):791-8.

52. Proch V, Singh BB, Schemann K, Gill JPS, Ward MP, Dhand NK. Risk factors for occupational *Brucella* infection in veterinary personnel in India. Transbound Emerg Dis. 2018;65(3):791-8.

53. Al-Aska AK, Chagla AH. Laboratory-acquired brucellosis. Journal of Hospital Infection. 1989;14(1):69-71.

54. Čekanac R, Mladenović J, Ristanović E, Lazić S. Epidemiological characteristics of brucellosis in Serbia, 1980-2008. Croatian Medical Journal. 2010;51(4):337-44.

55. Compes Dea C, Bescos JG, Agreda JPAP, Alvaro PMM, Blasco Martinez JM, Villuendas Uson MC. Epidemiological investigation of the first human brucellosis case in Spain due to *Brucella suis* biovar 1 strain 1330. Enfermedades infecciosas y microbiologia clinica. 2017;35(3):179-81.

56. Demirdal T, Demirturk N. Laboratory-acquired brucellosis. Annals Academy of Medicine. 2008;37(1).

57. Ergonul O, Celikbas A, Tezeren D, Guvener E, Dokuzoguz B. Analysis of risk factors for laboratory-acquired *Brucella* infections. The Journal of hospital infection. 2004;56(3):223-7.

58. Fiori PL, Mastrandrea S, Rappelli P, Cappuccinelli P. *Brucella abortus* infection acquired in microbiology laboratories. Journal of Clinical Microbiology. 2000;38(5):2005-6.

59. Gruner E, Bernasconi E, Galeazzi RL, Buhl D, Heinzle R, Nadal D. Brucellosis: an occupational hazard for medical laboratory personnel. Report of five cases. Infection. 1994;22(1):33-6.

60. Hartady T, Saad MZ, Bejo SK, Salisi MS. Clinical human brucellosis in Malaysia: a case report. Asian Pacific Journal of Tropical Disease. 2014;4(2):150-3.

61. Kiel FW, Khan MY. Brucellosis among hospital employees in Saudi Arabia. Infection control and hospital epidemiology. 1993;14(5):268-72.

62. Martin-Mazuelos E, Nogales MC, Florez C, Gomez-Mateos JM, Lozano F, Sanchez A. Outbreak of *Brucella melitensis* among microbiology laboratory workers. Journal of Clinical Microbiology. 1994;32(8):2035-6.

63. Memish ZA, Mah MW. Brucellosis in laboratory workers at a Saudi Arabian hospital. American Journal of Infection Control. 2001;29(1):48-52.

64. Noviello S, Gallo R, Kelly M, Limberger RJ, DeAngelis K, Cain L, et al. Laboratory-acquired brucellosis. Emerging infectious diseases. 2004;10(10):1848-50.

65. Ozaras R, Celik AD, Demirel A. Acute hepatitis due to brucellosis in a laboratory technician. European Journal of internal Medicine. 2004;15(4):264.

66. Rodrigues ALC, Silva SKL, Pinto BLA, Silva JB, Tupinambas U. Outbreak of laboratory-acquired *Brucella abortus* in Brazil: a case report. Rev Soc Bras Med Trop. 2013;46(6):791-4.

67. Sam IC, Karunakaran R, Kamarulzaman A, Ponnampalavanar S, Syed Omar SF, Ng KP, et al. A large exposure to *Brucella melitensis* in a diagnostic laboratory. Journal of Hospital Infection. 2012;80(4):321-5.

68. Smith JA, Skidmore AG, Andersen RG. Brucellosis in a laboratory technologist. Canadian Medical Association Journal. 1980;122.

69. Staszkiewicz J, Lewis CM, Colville J, Zervos M, Band J. Outbreak of *Brucella melitensis* among microbiology laboratory workers in a community hospital. Journal of Clinical Microbiology. 1991;29(2):287-90.

70. Wallach JC, Ferrero MC, Victoria Delpino M, Fossati CA, Baldi PC. Occupational infection due to *Brucella abortus* S19 among workers involved in vaccine production in Argentina. Clinical Microbiology and Infectious Diseases. 2008;14(8):805-7.

71. Wallach JC, Giambartolomei GH, Baldi PC, Fossati CA. Human infection with M-strain of *Brucella canis*. Emerging infectious diseases. 2004;10(1):146-8.

72. Yagupsky P, Peled N, Riesenberg K, Banai M. Exposure of hospital personnel to *Brucella melitensis* and occurrence of laboratory-acquired disease in an endemic area. Scandinavian journal of infectious diseases. 2000;32(1):31-5.

73. CDC. *Brucella suis* infection associated with feral swine hunting - three states, 2007-2008. MMWR Morbidity and Mortality Weekly Report. 2009;58(22):618-21.

74. Gelfand MS, Cleveland KO, Buechner D. Neurobrucellosis in a hunter of feral swine. Infectious Diseases in Clinical Practice. 2014;22(4):e103-e4.

75. Godfroid J, Al Dahouk S, Pappas G, Roth F, Matope G, Muma J, et al. A “One Health” surveillance and control of brucellosis in developing countries: Moving away from improvisation. Comparative Immunology, Microbiology and Infectious Diseases. 2013;36(3):241-8.

76. Ragan VE. The Animal and Plant Health Inspection Service (APHIS) brucellosis eradication program in the United States. Veterinary Microbiology. 2002;90(1–4):11-8.

77. Nishi JS, Stephen C, Elkin BT. Implications of agricultural and wildlife policy on management and eradication of bovine tuberculosis and brucellosis in free-ranging wood bison of northern Canada. Annals of the New York Academy of Sciences. 2002;969:236-44.

78. Bell JC, Palmer SR. Control of zoonoses in Britain: past, present, and future. British medical journal (Clinical research ed). 1983;287(6392):591-3.

79. Franc KA, Krecek RC, Hasler BN, Arenas-Gamboa AM. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. BMC Public Health. 2018;18(1):125.

80. Al Dahouk S, Tomaso H, Nöckler K, Neubauer H, Frangoulidis D. Laboratory-based diagnosis of brucellosis: a review of the literature. Part I: techniques for direct detection and identification of *Brucella* spp. Clinical Laboratory. 2003;49(9-10):487-505.

81. Al Dahouk S, Tomaso H, Nockler K, Neubauer H, Frangoulidis D. Laboratory-based diagnosis of brucellosis: a review of the literature. Part II: serological tests for brucellosis. Clinical laboratory. 2003;49(11-12):577-89.

82. Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. The Lancet Infectious Diseases. 2007;7(12):775-86.

83. Young EJ. An overview of human brucellosis. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 1995;21(2):283-9; quiz 90.

84. Pessegueiro P, Barata C, Correia J. Brucelose - uma revisão sistematizada. Medicina Interna. 2003;10(2):91-100.

85. Vered O, Simon-Tuval T, Yagupsky P, Malul M, Cicurel A, Davidovitch N. The Price of a Neglected Zoonosis: Case-Control Study to Estimate Healthcare Utilization Costs of Human Brucellosis. 2015. e0145086 p.

86. Young JE, Corbel JM. Brucellosis: clinical and laboratory aspects. 1989.

87. Dorneles EMS, Sriranganathan N, Lage AP. Recent advances in *Brucella abortus* vaccines. Veterinary Research. 2015;46(1):76.

88. Biosafety in microbiological and biomedical laboratories: Fifth edition, revised Dec. 2009. [Washington D.C.] : U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health. 2009.

89. Yagupsky P, Baron EJ. Laboratory exposures to brucellae and implications for bioterrorism. Emerging Infectious Diseases. 2005;11(8):1180-5.

S1 Appendix: PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
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.	4
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.	4
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.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	s3 appendix
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).	5
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.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
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.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

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).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
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.	s4 appendix
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.	s5 appendix
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).	s5 appendix
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.	6 - 10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	s5 appendix
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
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).	10 - 12
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).	10 - 12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12 - 13
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.	13

S2 Appendix: Extensive overview of search terms

CABI:

(veterinar* OR laboratorist* OR farmer* OR abattoir* OR slaughter* OR vaccinator* OR cowboy* OR student* OR butcher*) AND (expos*) AND (occupation* OR job-relat* OR professional* OR work*) AND (brucel* OR "malta fever" OR "Gibraltar fever" OR "bang’s disease")

Cochrane:

(veterinar* OR laboratorist* OR farmer* OR abattoir* OR slaughter* OR vaccinator* OR cowboy* OR student* OR butcher*) AND (expos*) AND (occupation* OR job-relat* OR professional* OR work*) AND (brucel* OR "malta fever" OR "Gibraltar fever" OR "bang’s disease")

Pubmed:

((((veterinar* OR laboratorist* OR farmer* OR abattoir* OR slaughter* OR vaccinator* OR cowboy* OR student* OR butcher*)) AND expos*) AND (occupation* OR job-relat* OR professional* OR work*)) AND (brucel* OR “malta fever” OR “Gibraltar fever” OR “bang’s disease”)

Science Direct:

(veterinar* OR laboratorist* OR farmer* OR abattoir* OR slaughter* OR vaccinator* OR cowboy* OR student* OR butcher*) AND (expos*) AND (occupation* OR job-relat* OR professional* OR work*) AND (brucel* OR "malta fever" OR "Gibraltar fever" OR "bang’s disease")

Scielo:

(veterinar* OR laboratorist* OR farmer* OR abattoir* OR slaughter* OR vaccinator* OR cowboy* OR student* OR butcher*) AND (expos*) AND (occupation* OR job-relat* OR professional* OR work*) AND (brucel* OR "malta fever" OR "Gibraltar fever" OR "bang’s disease")

Scopus:

ALL (veterinar* OR laboratorist* OR farmer* OR abattoir* OR slaughter* OR vaccinator* OR cowboy* OR student* OR butcher*) AND ALL (expos*) AND ALL (occupation* O R job-relat* OR professional* OR work*) AND ALL (brucel* OR malta AND fever OR gibraltar AND fever OR bangs AND disease)

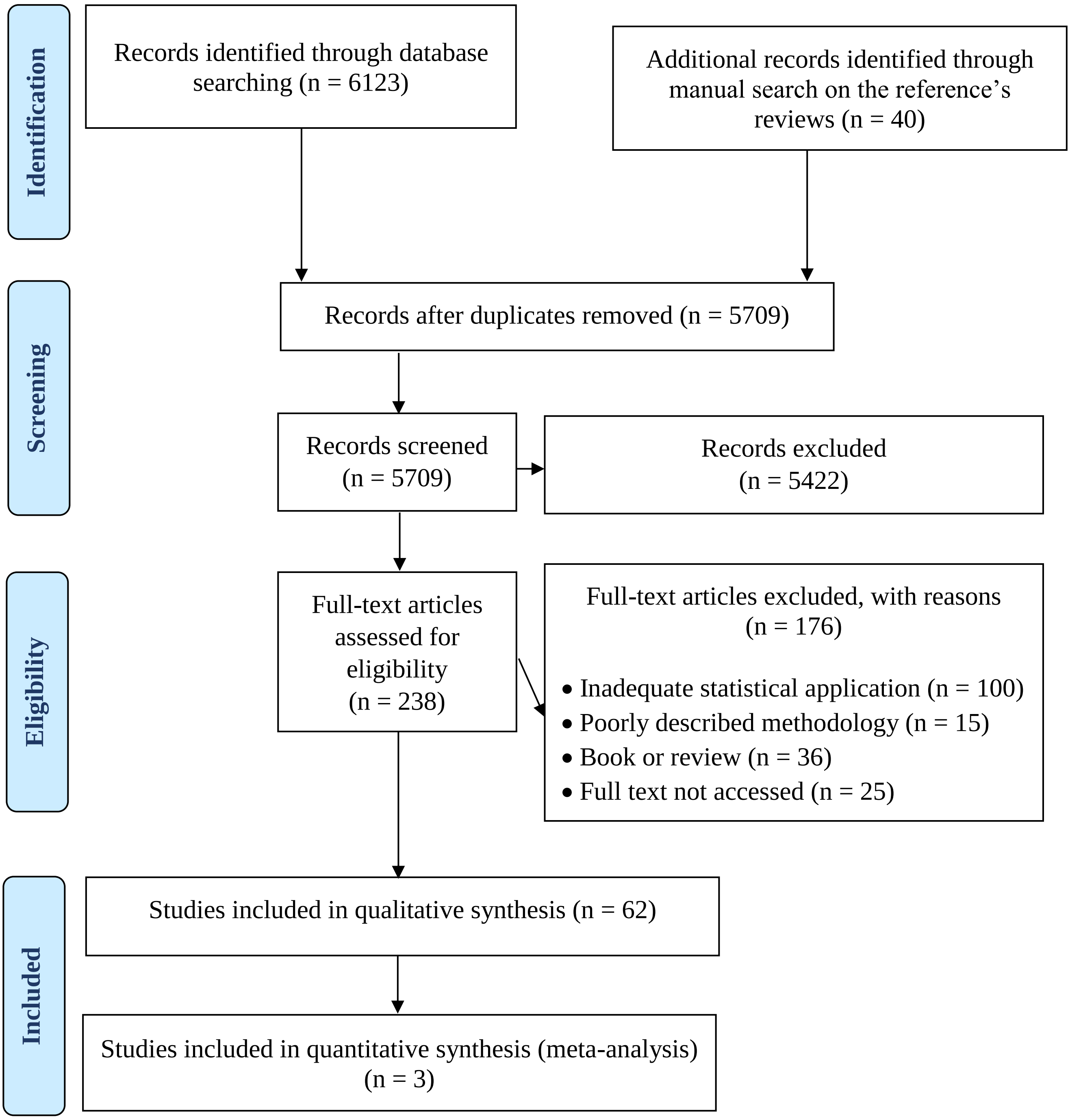
Web of Science:

TS=((veterinar* OR laboratorist* OR farmer* OR abattoir* OR slaughter* OR vaccinator* OR cowboy* OR student* OR butcher*)AND(expos*)AND(occupation* OR job-relat* OR professional* OR work*)AND(brucel* OR malta fever OR Gibraltar fever OR bangs disease))

S3 Appendix: Inclusion and exclusion criteria for selection of articles

Inclusion criteria	Exclusion criteria
• All countries • All years • *Brucella* spp. • Occupational exposure to brucellosis	• Epidemiological data about animals • Diagnosis of infection in animals • Genetics • Microbiology • Immunology • Molecular biology • Diagnostic performance of tests • Vaccination • Therapeutics • Languages other than English, Spanish, French or Portuguese • Full text not available

S4 Appendix: Flow diagram



S5 Appendix: Studies describing occupational context of *Brucella* spp. human infection

First author	Country	Time period	Positive/ Tested	Study design	Diagnostic	*Brucella* species	Predictors of transmission	Molecular link
Akhvlediani	Georgia	1970 – 1973 1988 – 1889 2004 – 2008	300/300	Case series	Criterion: Wright and Huddelson agglutination tests, combined with clinical signs. Cut off: titer of 1:200 or higher for Wright and Huddelson agglutination tests.	Not reported	Occupation: 29.0% (87) were shepherds and 12.3% (37) farmers. Animal contact: 86.3% (259) of all the patients, 40.4% (121) with sheep and 35.0% (105) with both sheep and cattle. Vaccine contact: 0.5% (1) patient reported exposure.	Not reported
Al Dahouk	Germany	1962 – 2005	6269/6269	Case series	Criterion: positive culture or standard tube agglutination test (STA) or complement fixation test (CFT) or ELISA, combined with the occurrence of an acute febrile illness or two other clinical signs. Cut off: only one significant titer (not described), or an increase in the titer in the follow-up serum sample.	*B. abortus* *B. melitensis* *B. suis*	Occupation: among the 102 cases in which the probable source of infection could be identified, 6.9% (7) were laboratory technician, 3.9% (4) shepherds, 2.0% (2) farmers, 3.9% (4) butchers and 1.0% (1) veterinarian. Animal contact: 15.7% (16) had direct contact with cattle, 23.5% (24) with sheep and 15.7% (16) with goats.	Not reported
Al-Aska	Saudi Arabia	Not reported	4/4	Case report	Criterion: positive culture or saline agglutination method combined with clinical signs. Cut off: not described, although the minimum titer reported was 1:1280 for saline agglutination method.	*B. melitensis*	Occupation: 100.00% (4) were laboratory workers. Positive culture contact: 25.0% (1) had mucous membrane contact due to a splash of contaminated solution on the face, 50.0% (2) had inhaled contaminated aerosols and 25.0% (1) had a needlestick injury, from a needle containing contaminated synovial fluid	Not reported
Al-Shamahy	Yemen	1992 – 1993	235/469	Case control	Criterion: positive culture or STA combined with clinical signs. Cut off: titer of 1:160 or higher for STA.	*B. melitensis*	Occupation: among the 235 cases, 38.3% (90) were farmers, 6.0% (14) shepherds, 6.0% (14) laboratory workers and 1.3% (3) abattoir workers.	Not reported
Ari	Kenya	2005	9/12	Case report	Criterion: positive rose bengal test (RBT) or CFT or STA or *Brucella* microagglutination test or rapid ELISA. Cut off: titer higher than 1:160 for STA and *Brucella* microagglutination test, and 1:320 for rapid ELISA.	Not reported	Occupation: among the tested individuals, 100.00% (12) were pastoralists, of which 16.7% (2) tested positive for RBT and 58.3% (7) for CFT. From 10 pastoralists tested with rapid ELISA and *Brucella* microagglutination test, were 80.0% (8) and 50.0% (5) were positive respectively.	Not reported

Arlett	Britain	1995	1/1	Case report	Criterion: positive direct agglutination and ELISA IgM and IgG, and dye test for *Brucella*. Cut off: not reported.	*B. melitensis*	Occupation: 100.00% (1) was a veterinary, who contracted the condition while working on the products of conception from animals.	Not reported
Ashford	United States of America	1998 – 1999	26/26	Case series	Criterion: clinical signs and self-report exposure, with or without positive culture. Cut off: not available, as RB51 do not induces immune response.	*B. abortus* – RB51 strain	Occupation: 80.8% (21) were veterinarians, 7.7% (2) veterinary students, 7.7% (2) veterinary technicians and 3.8% (1) ranch employee. Vaccine contact: 80.1% (21) had needle stick injuries, 15.4% (4) spray from the vaccine on the conjunctiva and 3.8% (4) spray from the vaccine on broken skin.	*B. abortus* strain RB51 was isolated from the surgical wound of a case 3 days after inoculation.
Asiimwe	Uganda	2013	45/90	Case control	Criterion: positive STA. Cut off: titers of 1:160 or higher for STA.	Not reported	Occupation: 11.1% (5) were farmers and 88.9% (40) agropastoralists. Animal contact: 42.2% (19) rear goats and sheep.	Not reported
Avdikou	Greece	2002 – 2004	152/152	Case series	Criterion: positive STA, or RBT or ELISA or positive blood culture, and/or clinical signs and/or clinician's decision for anti-brucellosis treatment. Cut off: titers higher than 1:320 for STA.	Not reported	Occupation: 60.5% (92) were shepherds, 1.3% (2) veterinarians or assistants, 2.6% (4) abattoir workers and 0.6% (1) tannery worker. Animal contact: 75.0% (114) had direct contact with animals. Vaccine contact: 25.0% (41) reported contact with the REV-1 vaccine. Animal fluids contact: 36.8% (56) had contact with an aborted fetus.	Not reported
Aworh	Nigeria	2010 – 2011	54/224	Sectional	Criterion: positive RBT or ELISA. Cut off: not reported.	*B. abortus* and *B. melitensis*	Occupation: among the 54 cases, 59.2% (32) were butchers, 20.4% (11) meat sellers and 14.8% (8) abattoir cleaners. Animal fluids contact: among the 54 cases, 53.7% (29) of them reported handling aborted fetus, 64.8% (35) slaughtering animals and 63.0% (34) slaughtering animals with an injury. Personal protective equipment: among the 54 cases, 96.3% (52) of them reported not wearing gloves.	Not reported
Blasco and Díaz	Spain	Not reported	2/2	Case report	Criterion: positive culture or STA or Coombs' test or RBT. Cut off: not described, although the minimum titer reported was 1:320 for STA and 1:1280 for Coombs' test.	*B. melitensis* – REV-1 strain	Occupation: 100.00% (2) were veterinarians. Vaccine contact: 100.00% (2) had a previous contact with REV-1 vaccine and 50.0% (1) had an accidental self-inoculation.	Not reported

Bosilkovski	Macedonia	1989 – 1990 2000 – 2001 2011 – 2014	340/340	Case series	Criterion: RBT was performed in all patients. Depending on the time period, antibody titers were determined by the STA plus *Brucella* Coombs' test (in the first two periods) or by the immunocapture assay (in the third period), combined with clinical signs. Cut off: titer of 1:160 or higher for STA, 1:320 for Coombs' test and 1:640 for immunocapture assay.	Not reported	Occupation: 6.5% (22) were veterinarians or veterinary technician. Animal contact: 63.8% (217) had reported contact with animals.	Not reported
Bourne	Canada	1963	3/3	Case report	Criterion: positive culture or STA. Cut off: titers higher than 1:320 for STA, although the minimum titer reported was 1:1280.	*B. abortus*	Occupation: 100.00% (3) were employed by or around a packing house; 33.3% (1) was a truck driver, 33.3% (1) work constructor and 33.3% (1) slaughter-house employee. Animal fluids contact: 33.3% (1) handled discarded carcasses of animals, 33.3% (1) worked as a construction worker 20 feet from a pile of animal carcasses, in a packing plant, and 33.3% (1) worked as a slaughter-house employee.	Not reported
Campbell	Vietnam	2016 – 2017	10/10	Case report	Criterion: positive test on culture and identified as *Brucella* spp. in a VITEK2 system. Cut off: no serology was performed.	*B. melitensis*	Occupation: 10.0% (1) was veterinarian. Animal contact: 100.00% (10) reported exposure to goats prior to the febrile episodes; 80.0% (8) kept goats and 20.0% (2) had consumed goat meat. Vaccine contact: 10.0% (1) vaccinated goats.	Not reported
Cash-Goldwasser	Tanzania	2012 – 2014	50/562	Cohort	Criterion: positive culture or *Brucella* microagglutination test, combined with clinical signs. Cut off: a four-fold or higher rise in *Brucella* antibody titer, between acute and convalescent serum samples. Probable cases were defined as a single titer of 1:160 or higher for *Brucella* microagglutination test.	Not reported	Occupation: among the 50 cases, 30.0% (15) were farmers and 2.0% (1) livestock attendant. Animal contact: 6.0% (3) assisted livestock abortions, 8.0% (4) assisted livestock births, 18.0% (9) cleaned livestock waste and 14.0% (7) slaughtered livestock.	Not reported
CDC	United States of America	2007 – 2008	3/3	Case report	Criterion: positive culture and PCR combined, or *Brucella* microagglutination test with clinical sign.	*B. suis*	Occupation: 100.00% (3) were hunters. Animal fluid contact: 33.3% (1) cut his hand with a knife while field dressing one of the hunted feral swine.	A frozen sausage and tenderloin of a feral

					Cut off: titers of 1:640 or higher for *Brucella* microagglutination test.		Personal protective equipment: 66.6% (2) of the 3 hunters adopted no individual protection measure during the handling of feral swine.	swine hunted by two patients was positive for *B. suis* isolation, and had identical MLVA signatures compared with *B. suis* isolated from one of the patients.
Čekanac	Serbia	1980 – 2008	1521/1521	Case series	Criterion: positive STA, ELISA, CFT, Coombs' test, or fluorescent antibody test, combined with clinical signs. Cut off: not reported.	Not reported	Occupation: among 70 infected patients, from 1999 to 2006, 4.3% (3) were laboratory workers. Animal contact: among 47 outbreaks, from 1991 to 2008, 30.0% (14) had contact with infected animals, which was considered the major mode of transmission. Among 70 infected patients, from 1999 to 2006, 50.0% (35) had direct or indirect contact with infected sheep, 4.3% (3) with infected goats and 15.7% (11) with other infected animals. Positive culture contact: among 70 infected patients, from 1999 to 2006, 4.3% (3) were in contact with specimens from infected animals and 1.4% (1) was exposed accidentally during specimen collection.	
Compés Dea	Spain	2014	1/1	Case report	Criterion: positive culture and PCR combined with clinical signs. Cut off: no serology was performed.	*Brucella suis* – biovar 1 strain 1330	Occupation: 100.00% (1) worked in a medical waste treatment plant. Contact with sharp object: 100.00% (1) poked his foot with a needle that was lying on the floor. Personal protective equipment: although properly used, was not enough to prevent transmission.	An MLVA analyze was performed from the isolated strain and an identical pattern with the reference *B. suis* biovar 1 strain 1330 was observed.
Cooper	Saudi Arabia	1988	150/300	Case control	Criterion: positive culture or STA, and 2-mercaptoethanol	Not reported	Occupation: among the 150 cases, 56.6% (87) were owner of animals.	Not reported

					(2-ME) or Coombs' test, all combined with clinical signs. Cut off: titers of 1:1280 or higher for STA, 2-ME or Coomb's test.		Animal contact: among the 150 cases, 74.0% (111) had direct contact with animals; 0.6% (1) cows, 8.0% (12) camels, 42.0% (63) sheep and 23.3% (35) goats. Among 150 cases, 38.6% (58) slaughtered animals and 31.3% (47) helped animal parturition.	
Dean	Togo	2011	7/683	Sectional	Criterion: positive for RBT or ELISA. Cut off: not reported	Not reported	Occupation: among the 7 cases, 85.7% (6) were from pastoralists communities.	Not reported.
Demirdal	Turkey	Not reported	3/3	Case report	Criterion: positive culture or RBT, STA or biochemical reactions, combined with clinical signs. Cut off: not described, although the minimum titer reported was 1:640 for STA.	*B. melitensis*	Occupation: 100.00% (3) were laboratory workers. Positive culture contact: no accident occurred. Although using masks, transmission was probably due to aerosol contamination because of the current practice of sniffing culture plates. 100.00% (3) of the professionals from the *Brucella* cases were found to be working on the specimen of the index case patient.	Not reported
Ergonul	Turkey	2000 - 2003	12/12 & 7/55	Case control	Criterion: clinical illness (characterized by acute or insidious onset of fever, night sweats, undue fatigue, anorexia, weight loss, headache, and arthralgia), with isolation of *Brucella spp*. (positive blood culture) from clinical specimens or STA. Cut off: titer of 1:320 of higher for STA.	*B. melitensis* – biovar 1 and 3	Occupation: 100.00% (12) were healthcare workers; 50.0% (6) were physicians, 8.0% (1) was a laboratory technician, 8.0% (1) was a nurse, 17.0% (2) were secretaries and 17.0% (2) were staff disposing contaminated laboratory material. Probable reason for infection: 58.0% (7) were processing cultures, 16.0% (2) were disposing of laboratory material and 26.0% (3) were eating and drinking near the microbiology bench. Risk factor analysis (univariate analysis): both male and female physicians working in bacteriology laboratories had a significantly higher risk for Brucella infection (P = 0.021 and P = 0.041, respectively), whereas the age of the healthcare workers, duration of work (experience) and recent work with Brucella bacteria were not significant risk factors. The use of gloves always or sometimes was more protective than non-use of it (P = 0.005). Risk factor analysis (multivariate analysis): male physicians had a higher risk of Brucella infection (P = 0.008), and using gloves was found to be protective (P = 0.017).	
Fiori	Italy	1990 – 1991	12/12	Case report	Criterion: positive RBT, microagglutination test or STA.	*B. abortus*	Occupation: 100.00% (12) laboratory workers. Contact with positive culture: 100.00% (12) of cases worked in the same laboratory where the	The original *B. abortus* biotype 1

					Cut off: not described, although the minimum titer reported was 1:320 for STA.		outbreak occurred. It originated from the accidental rupture of a polystyrene centrifuge tube containing *B. abortus* biotype 1 atypical strain (previously isolated from a camel), during transfer of the tube from one room to another.	strain was obtained from blood samples of all the three infected individuals.
Gelfand	United States of America	Not reported	1/1	Case report	Criterion: positive culture and serology for *Brucella* (not specified) combined with clinical signs. Cut off: not described, although the minimum titer reported was 1:160 for IgM and 1:1280 for IgG.	*B. suis*	Occupation: 100.00% (1) hunters. Animal fluids contact: 100.00% (1) hunted, field-dressed, and butchered wild pigs several weeks before the onset of illness.	Not reported
Gonçalves	Brazil	2007	3/207	Sectional	Criterion: positive tamponed acidified antigen test was confirmed with a 2-ME. Cut off: not described, although the minimum titer reported was 1:50 for 2-ME.	Not reported	Occupation: 100.00% (3) rural residents and owner of animals. Animal fluid contact: 66.6% (2) had performed artificial insemination.	Not reported
Gruner	Switzerland	1990 – 1991	5/5	Case report	Criterion: positive culture or *Brucella* microagglutination test, IgM and IgG ELISA or CFT combined or not with clinical signs. Cut off: not described, although the minimum titer reported was 1:160 for *Brucella* microagglutination test.	*B. melitensis*	Occupation: 100.00% (5) laboratory workers. Positive culture contact: 40.0% (2) had worked with strains of *Brucella* (isolated from index case I); 60.0% (3) had worked with strains of *Brucella* (isolated from index case II). Protective personal equipment: all the index cases were handled without any specific safety precautions, such as work under a safety hood, wearing of gloves, masks and/or protective glasses.	Conventional typing was unable to distinguish between the isolates and all were identified as *B. melitensis* biovar 3.
Guney	Turkey	2007	1/1	Case report	Criterion: positive cultured and *Brucella* agglutination test combined with clinical signs. Cut off: not described, although the minimum titer reported was 1:320 for *Brucella* agglutination test.	*B. melitensis*	Occupation: 100.00% (1) farmer.	Not reported
Hartady	Malaysia	2013	1/1	Case report	Criterion: positive PCR, RBT and STA combined with clinical sings. Cut off: not described, although the minimum titer	*B. melitensis*	Occupation: 100.00% (1) laboratory worker. Positive culture contact: 100.00% (1) were involved in a research on isolation of *B. melitensis* from goats, for the past 3 months.	Not reported

					reported was +++ for RBT and 1:40 for STA.			
Hasanjani Roushan	Iran	1997 – 2003	469/469	Case series	Criterion: positive STA or 2-ME combined with clinical signs. Cut off: titers of 1:320 or higher for STA and 1:160 for 2-ME.	Not reported	Occupation: 11.3% (53) animals' breeders, 1.5% (7) veterinarians and 8.1% (38) laboratory workers.	Not reported
Hendricks	United States of America	1959 – 1960	128/1627	Case series	Criterion: positive culture or blood agglutination test combined or not with clinical signs. Cut off: not described, although the minimum titer reported was 1:160 for blood agglutination test.	*B. suis* and *B. melitensis*	Occupation: among the 128 cases, 100.00% (128) were employees of a swine-slaughtering plant. Animal fluids contact: the employees were classified by labor department, and the percentages of positives were: 33.7% (60) of 178 from killing, 12.2% (24) of 197 from cutting, 24.4% (20) of 82 from casing, 7.4% (4) of 54 from head trimming, 5.3% (3) of 57 from the freezer, 9.4% (3) of 32 from inedible, 4.3% (3) of 69 from the maintenance room, 4.4% (2) of 45 from the pork plant, 4.3% (2) of 46 from curing, 1.9% (1) of 54 from boning, 2.2% (1) of 45 from night cleaning-up, 0% (0) of 450 from other departments, 1.0% (3) of 300 from management and office, 18.2% (2) of 11 from killing inspection and 0% (0) of 7 from other inspection sectors.	The isolation of *B. suis* from the air at two locations on the killing floor demonstrates that one of the etiologic agents was present in the air during the outbreak.
Jia	China	2001 – 2005	10/10	Case report	Criterion: positive culture or STA combined with clinical signs. Cut off: titers of 1:100 or higher for STA.	*B. melitensis*	Occupation: 50.0% (5) farmers, 30.0% (3) herdsmen, 10.0% (1) teacher and 10.0% (1) student who lived in rural areas. Animal contact: 70.0% (7) had a history of close contact with cattle and sheep.	Not reported
Joffe	United States of America	Not reported	1/1	Case report	Criterion: positive culture and STA combined with clinical signs. Cut off: not described, although the minimum titer reported was 1:80 for blood agglutination test.	*B. abortus* – S19 strain	Occupation: 100.00% (1) veterinarian. Vaccine contact: 100.00% (1) accidentally injected the *B. abortus* – S19 vaccine strain into the skin, between the hand palm and the right thumb.	*B. abortus* – S19 strain was cultured from discharge at the injection site obtained on the eighth day after self-inoculation.
Kiel	Saudi Arabia	1983 – 1990	9/9	Case report	Criterion: positive culture or *Brucella* serology combined with clinical sings.	*B. melitensis*	Occupation: 100.00% (9) hospital employees; 77.8% (7) bacteriology technologists, 22.2% (2) nurses and 11.1% (1) obstetrician.	Not reported

					Cut off: not described, although the minimum titer reported was 1:1280 for *Brucella* serology.		Positive culture contact: 11.1% (1) recalled sniffing next to bacteriology plates, as part of his diagnostic approach, and 11.1% (1) removed culture plates from the safety hood, for closer visual examination. Positive patient assistance: 11.1% (1) disclosed that he occasionally participated in precipitous deliveries when wearing gloves was not possible; 11.1% (1) probably had contact with the body fluids of one of the 76 brucellosis patients hospitalized.	
Kozukeev	Kyrgyzstan	2003	100/200	Case control	Criterion: positive Wright agglutination test combined with clinical signs. Cut off: titers higher than 1:100 in Wright agglutination test.	Not reported	Occupation: among the 100 cases, 12.0% (12) were collective farm workers and 86.0% (86) reported owning farm animals at home, of which 80.0% (80) were cattle, 70.0% (70) goats, 50.0% (50) dogs and 49.0% (49) sheep. Animal contact: among 86 cases that reported having farm animals at home, 81.3% (70) cleaned barns, 69.7% (60) assisted in animal delivery and 25.6% (22) slaughtered animals.	Not reported
Mailles	France	2004 – 2013	250/250	Case series	Criterion: positive culture or PCR or a four-fold or greater rise in *Brucella* antibody titers between acute and convalescent phase serum (RBT, Wright test, competitive ELISA, Brucellacapt lateral flow immunochromatography and indirect immunofluorescence for IgM and IgG detection). Cut off: not reported.	*B. suis* and *B. melitensis*	Occupation: among the 250 cases, 85.2% (213) were imported, of which 6.1% (13) had a risk occupation while staying in the endemic country, considering that 5.2% (11) were cattle breeders, 0.5% (1) veterinarian and 0.5% (1) agronomist; 14.8% (37) were domestic, of which 45.9% (17) involved laboratory workers with occupational brucellosis. Animal contact: among the 213 imported cases, 53.1% (113) had direct contact with animals that could be a possible source of Brucella, these being 21.1% (45) sheep, 17.8% (38) goats and 14.1% (30) cows; among 250 cases, 14.8% (37) were domestic, of which 10.8% (4) had never traveled out of the country, but lived in cattle farms before the country was declared officially free of brucellosis, and 2.7% (1) of the domestic cases had reported an episode of brucellosis in the herd.	An index case could be identified for 94.1% (16) of the laboratory cases, and was for all of them an imported case. In most cases, identity of respective paired strains at molecular level, trough MLVA16.
Mamani	Iran	2014 – 2015	29/218	Sectional	Criterion: positive STA retested with Coombs' and Wright test, and negative STA retested with 2-ME.	Not reported	Occupational: among 29 cases, 16.9% (19) of 112 were butchers, 8.1% (7) of 86 were slaughterhouse workers and 15.0% (3) of 20 were veterinarians.	Not reported

					Cut off: titers of 1:80 or higher for Wright test.		Personal protective equipment: among 112 butchers, 64.3% (72) used masks, 14.3% (16) goggles, 4.5% (5) gloves, 33.9% (38) boots and 42.9% (48) apron. Among 86 slaughterhouse workers, 11.6% (10) used masks, 4.7% (4) goggles, 23.3% (20) gloves, 87.2% (75) boots and 61.6% (53) apron. Among 20 veterinarians, 60.0% (12) used masks, 35.0% (7) goggles, 60.0% (12) gloves, 30.0% (6) boots and 45.0% (9) apron.	
Martin-Mazuelos	Spain	1998	4/4	Case report	Criterion: positive culture and RBT plate, along with Coombs' and microagglutination test, all combined with clinical signs. Cut off: not described, although the minimum titer reported was 1:40 for the initial agglutinin.	*B. melitensis*	Occupation: 100.00% (4) laboratory workers. Positive culture contact: 100.00% (4) handled blood cultures. No accident occurred in the laboratory at that time, and the blood cultures were handled correctly, except that a biosafety hood was not used.	Not reported
Memish	Saudi Arabia	1991 – 2000	7/7	Case report	Criterion: positive culture or STA combined with clinical signs. Cut off: titers of 1:320 or higher for STA, although all cases had a titer of 1:1280 or higher.	*B. melitensis* and *B. abortus*	Occupation: 100.00% (7) laboratory workers. Positive culture contact: 85.7% (6) worked with positive *Brucella* cultures and 14.3% (1) sniffed and handled, outside of the biosafety cabinet, a culture of *Brucella* erroneously identified as gran positive; 14.3% (1) visited a laboratory where *Brucella* cultures were tested.	Not reported
Mousa	Kuwait	1984 – 1985	379/379	Case series	Criterion: Positive culture or STA or immunofluorescence test. Cut off: titer higher than 1:160 for tube agglutination test, higher than 1 :640 for immunofluorescent or a four-fold rise in titers.	*B. melitensis*	Occupation: 39.8% (151) students, 24.3% (92) housewives, 7.7% (29) retired, 6.3% (24) shepherds, 7.7% (29) unemployed, 5.5% (21) soldiers, 4.5% (17) civil servants, 2.1% (8) skilled workers, 1.3% (5) traders and 0.5% (2) farmers and 0.3% (1) engineer. Animal contact: 11.1% (42) reported direct contact with animals and denied consuming raw milk.	Not reported
Mufinda	Angola	2012	39/323	Sectional	Criterion: positive STA or RBT. Cut off: titers higher of 1:160 for STA combined with positive result for RBT.	Not reported	Occupation: among the 39 cases, 17.9% (7) of 131 were slaughterhouse workers and 82.1% (32) of 192 were cattle breeders. Animal handling: among the 32 cattle breeders, 78.1% (25) reported that abortion remains were abandoned in the pasture and eventually ingested by dogs and pigs	Not reported
Nicoletti	United States of America	1984	1/1	Case report	Criterion: positive STA combined with clinical sings. Cut off: not described, although it was reported an	B. abortus – S19 strain	Occupation: 100.00% (1) veterinary student. Vaccine contact: 100.00% (1) accidentally injected himself in the third left finger, during vaccination of a calf.	Not reported.

					eight-folder titer to 1:160 a month, after the accident.			
Noviello	United States of America	2001 – 2002	2/2	Case report	Criterion: positive culture and STA. Cut off: not described, although the minimum titer reported was 1:640 for STA.	*B. melitensis*	Occupation: 100.00% (2) laboratory workers. Positive culture contact: approximately two months before her illness, laboratory worker 2 had personally processed laboratory worker 1's blood culture, but had characterized the isolate as coryneform bacilli, in a class II biosafety cabinet.	
Ozaras	Turkey	Not reported	1/1	Case report	Criterion: positive culture and Wright test combined with clinical signs. Cut off: not described, although the minimum titer reported was 1:1280 for Wright test.	Not reported	Occupation: 100.00% (1) laboratory worker.	Not reported
Pisani	Uruguay	2009 – 2010	14/14	Case series	Criterion: positive RBT and ELISA IgM combined with clinical signs. Cut off: not described, although 85.7% (12) reported titers were 1:320 for ELISA IgM.	Not reported	Occupation: 100.00% (14) slaughtered workers. Animal fluid contact: 57.1% (8) of the cases worked at slaughter department, 7.1% (1) at viscera sector, 7.1% (1) at cattle shed, 14.3% (2) cleaning, 7.1% (1) at maintenance room and 7.1% (1) at tripe room. Personal protection equipment: 0% (0) used goggles.	Not reported
Proch	India	2015 - 2016	64/279	Sectional	Criterion: positive RBT, or STA and IgG ELISA. Cut off: titer of 80 international units or higher for STA, and optical density between 0.456 and 0.798 for IgG ELISA.	Not reported	Occupation: among 64 cases, 100.00% (64) were veterinarians. Vaccine contact: among 296 interviews, 4.1% (12) reported a *Brucella* needlestick injury. Animal contact: among the 296 individuals interviewed about the activities done a month before the research, 69.3% (205) had attended parturitions, 35.8% (106) handled aborted fetus, 64.5% (191) handled retained placenta, 44.6% (132) handled stillbirth, 75.0% (222) performed artificial insemination and 73.0% (216) attended infertility cases. Personal protective equipment: among the 296 individuals interviewed about the protection measures adopted a month before the research, 44.7% (130) did not use it while handling healthy animals, 28.1% (82) did not use it while handling sick animals, 84.8% (245) did not use it in parturition, 92.7% (265) did not use it while handling feces and urine, 88.3% (257) did not use it while handling aborted	Not reported

							fetus and stillbirth, and 87.7% (256) did not use it while handling retained placenta.	
Rodrigues	Brazil	2012	3/11	Case report	Criterion: positive ELISA IgM or IgG combined or not with clinical signs. Cut off: not reported.	*B. abortus*	Occupation: among the cases, 100.00% (3) were laboratory workers. Positive culture exposure: 100.00% (3) were inside the laboratory, were media seeded with *B. abortus* and manipulated in a damage biological safety cabinet. Personal protective equipment: 100.00% (3) reported wearing laboratory coats, disposable gloves and N95 masks regularly, when they were inside the laboratory.	Not reported
Rodríguez Valín	Spain	1998 – 1999	28/106	Case control	Criterion: positive culture or RBT, along with STA and Coombs' test, combined with clinical signs. Cut off: titers higher than 1:80 for STA and higher than 1:320 for Coomb's test.	*B. melitensis*	Occupation: 100.00% (106) slaughtered workers. Among the cases, 50.0% (14) worked at slaughtered department, 17.9% (5) at residues sector, 7.14% (12) at chamber, 3.6% (1) at cleaning, 7.14% (1) at maintenance room, 10.7% (3) at external companies and 3.6% at sanity services. Animal fluids contact: 46.4% (13) of the 28 cases reported cutting themselves with dirty sharps. Personal protective equipment: among the 106 cases, 32.1% (9) did not use gloves, 35.7% (10) did not use mask and 42.9% (12) did not use goggles.	Not reported
Sam	Malaysa	2009	4/51	Case report	Criterion: positive STA, RBT, Coombs' test and CFT. Cut off: titers of 1:80 or higher for STA.	*B. melitensis*	Occupation: 100.00% (51) laboratory workers. There were 52.9% (27) with high-risk exposure (handled cultures on the open bench or were within 1.5 m of such activities) and 47.1% (24) with low-risk exposure (were present in the laboratory at the time the processing was done, but further than 1.5 m from the *Brucella* cultures). Positive culture exposure: the cultures had been handled openly on the blood culture bench; the class II biosafety cabinet was not used and there was no directional airflow system in the laboratory. The bacterial suspension was made in a biosafety cabinet which had not been switched on, and the suspension was boiled in a closed tube on an open bench before DNA extraction. Among the cases, 100.00% (4) were classified as high-risk group.	Not reported

Smith	United States of America	1979	1/1	Case report	Criterion: positive culture and STA combined with clinical signs. Cut off: not described, although the minimum titer reported was 1: 1280 for STA.	*B. melitensis*	Occupation: 100.00% (1) laboratory worker. Positive culture exposure: the microbiologist had been working with positive blood cultures on an open bench.	Not reported.
Sofian	Iran	2005	150/300	Case control	Criterion: positive STA and 2-ME combined with clinical signs. Cut off: titers of 1:160 or higher for STA combined with the presence of 2-ME equal or higher than 20.	Not reported	Occupation: among the cases, 33.3% (50) were farmers, 0.7% (1) butcher, 5.3% (8) animal husbandry and 2.7% (4) farmers and animal husbandry. Animal contact: among the cases, 88.7% (133) had kept cattle, and 76.7% (102/133) had vaccinated their animals, although no question of accidental exposure has been studied.	Not reported
Staszkiewicz	United States of America	1988	8/75	Case report	Criterion: positive culture or STA combined with clinical signs. Cut off: titers of 1:160 or higher for STA.	*B. melitensis*	Occupation: among the cases, 100.00% (8) were laboratory workers. Positive culture exposure: 6 weeks before the first case of brucellosis occurred, a positive isolate was handled on an open workbench and not in a biologic safety cabinet. The original patient isolate and all employee isolates were identified at the Centers for Disease Control as *B. melitensis* biotype 3.	The original patient isolate and all employee isolates were identified as *B. melitensis*, biotype 3
Strbac	Serbia	2000 – 2014	102/102	Case series	Criterion: STA, Wright or BMAT (*Brucella* micro agglutination test). Cut off: not reported.	Not reported	Occupation: 22.5% (23) were agriculturist, 11.8% (12) were rearer, 10.8% (11) were veterinary, 6.9% (7) were cattleman and 2.9% (3) were stockbreeder. Animal contact: 43.1% (44) cases indicated daily direct contact with domestic animals.	Not reported
Tee	England	1969	1/1	Case report	Criterion: positive blood culture and saline agglutination test, along with CFT and 2-ME, all combined with clinical signs. Cut off: not described, although the minimum titer reported was 1: 320 for saline agglutination, less than 5 for CFT and 640 in 2-ME.	*B. abortus*	Occupation: 100.00% (1) farmer.	Not reported
Thomas	England	1991 – 1996	3/404	Cohort	Criterion: positive CFT and microagglutination test, or ELISA IgG and ELISA IgM.	Not reported	Occupation: among the cases, 100.00% (3) farmers.	Not reported.

					Cut off: titers of 80 or higher for CFT, 20 or higher for microagglutination and 80 or higher for ELISA IgG and ELISA IgM.			
Tsegay	Ethiopia	2013 – 2014	2/149 tested	Sectional	Criterion: RBT for screening and CFT for confirmation. Cut off: +++ on the RBT.	Not reported	Occupation: RBT were positive for 1.7% (1) of 60 slaughterers, 6.5% (2) of 31 loaders and 17.4% (4) of 23 cleaners. CFT confirmed 3.2% (1) loader and 4.4% (1) cleaner with positive diagnosis. Animal fluids contact: 48.7% (76) of 156 interviewed workers related accidentally cut during slaughtering and eviscerating. Personal protective equipment: among the 156 respondents, 81.4% (127) replied not using gloves and 86.6% (135) reported not covering their mouth during slaughtering and eviscerating process.	Not reported
Wallach	Argentina	1999 – 2006	21/30	Case series	Criterion: Positive for at least two tests STA, 2-ME, RBT or CFT, combined or not with clinical signs. Cut off: titers of 1:100 or higher for STA.	Not confirmed, but probably *B. abortus* – S19 strain	Occupation: 100.00% (21) workers from S19-manufacturing plants. Vaccine exposure: among all employees, 30.0% (9) related some kind of exposure; 20.0% (6) percutaneous, 10.0% (3) inhalatory and 3.3% (1) conjunctival (the individual had more than one route of exposure).	Not reported
Wallach	Argentina	Not reported	1/1	Case report	Criterion: positive culture combined with clinical signs.	*B. canis* – M strain	Occupation: 100.00% (1) laboratory worker (production of *B. canis* – M-strain used for serologic diagnosis). Positive culture exposure: 100.00% (1) handled a positive culture outside of a biological safety cabinet and related resuspension, by repeated pipetting with his mouth. Personal protective equipment: 100.00% (1) did not use any protection.	Not reported
Wallach	Argentina	2014 – 2015	17/17	Case series	Criterion: positive culture or RBT confirmed by STA, CFT and competitive ELISA, all combined with clinical signs. Cut off: titers of 1:100 or higher for STA, 10 for CFT or 28.0% of inhibition percentage for competitive ELISA.	*B. suis*	Occupation: RBT and STA were positive in 100.00% (17) patients. CFT and competitive ELISA were performed in 14 cases and were positive in 92.9% (13) of them. Blood cultures were positive in 82.3% (14) of the 17 patients. Among the cases, 100.00% (17) were workers from a pork processing plant. Information about work was available for 11 patients, of which 63.6% (7) worked in the killing area and 43.64% (4) worked at animal cleaning and transportation.	Not reported

							Personal protective equipment: 100.00% (17) used goggles, gloves and masks.	
Wallach	Argentina	Not reported	33/60	Case series	Criterion: positive culture or RBT, along with STA, 2-ME, and ELISA IgG and IgM. Cut off: titers higher than 50 IU/mL for STA and 2-ME; values of absorbance higher than 30 for ELISA.	*B. melitensis*	Occupation: among the cases, 75.8% (25) were rural workers, and among healthy individuals, 18.5% (5) were engaged in rural labor. Among 21 active patients who declared continuous contact with goats, 85.7% (18) developed rural activities, 9.5% (2) were masons and 4.8% (1) was a backer. Animal contact: among 23 individuals who reported continuous contact with goats, 91.3% (21) developed brucellosis, and only 32.0% (11) of 28 individuals who reported occasional contact became ill. Only 11.0% (1) of 9 individuals who reported no contact with goats developed brucellosis.	*B. melitensis* biovar 1 was isolated in from 17 milks and 11 colostrum samples obtained from goats, and from 42.4 % (14) among the 33 cases who lived close to the animals.
Williams	England	1968 – 1968	30/30	Case report	Criterion: positive phenol saline agglutination test, 2-ME, antihuman globulin test and CFT, all combined with clinical signs. Cut off: not described, although the minimum titer reported was 1:640 for saline agglutination, 1:320 for CFT, 1:5120 for antihuman globulin test and 1:1280 for 2-ME.	Not reported	Occupation: 70.0% (21) were farmers or farmworkers.	Not reported
Yagupsky	Israel	1997	7/7	Case report	Criterion: positive culture or positive RBT confirmed with STA and 2-ME, combined or not with clinical signs. Cut off: titers of 1:160 or higher for STA and 1:20 or higher for 2-ME.	*B. melitensis*	Occupation: 100.00% (7) were hospital personnel, who worked or visited a clinical microbiology laboratory. Positive culture exposition: a *Brucella* culture was misidentified as *Streptococcus* and manipulated outside the safety cabinet.	The biotyping of the isolates showed that the outbreak was caused by 3 different *B. melitensis*
Zhan	China	2005	3/14	Case report	Criterion: positive saline agglutination test combined with clinical signs. Cut off: not described, although the minimum titer reported was 1:800 for saline agglutination test.	Not reported	Occupation: 100.00% (3) were processing sheep placenta. Animal fluid contact: 100.00% (3) processed sheep placenta and reported they had already splashed animal fluids on their face. Personal protective equipment: 100.00% (3) used gauze mask and rubber glove, but did not wash their hands with disinfectant after work.	Not reported

CHAPTER 2: Formatted according to the submission guidelines of Preventive Veterinary Medicine

Occupational brucellosis among veterinarians in Minas Gerais state, Brazil

Abstract

Brucellosis is an important occupational disease, mainly among veterinarians, because of their frequent contact with sick animals, contaminated secretions and live attenuated anti-*Brucella* vaccines. This study aimed to determine the prevalence of accidental exposure to S19 and RB51 vaccine strains and occupational brucellosis among veterinarians registered to perform vaccination in Minas Gerais, Brazil, as well as to identify the risk factors associated with accidental exposure to anti-*Brucella abortus* vaccines. Data were collected by means of an online questionnaire. Three hundred and twenty nine veterinarians were included in the analyzes, using a stratified random sampling. A multivariable logistic regression analysis was used to evaluate the predictors of accidental exposure to S19 and RB51 strains. Nearly one-third, 32.83% (108/329) [95% confidence interval (CI): 27.78 to 38.19%] reported having been accidentally exposed to S19 or RB51 vaccine strains. The risk associated with this outcome included score of personnel protective equipment (PPE) use during work [odds ratio (OR), 0.94; 95% CI: 0.89 to 0.98] and score of knowledge about brucellosis symptoms, classified in mean (OR, 0.26; 95% CI: 0.07 to 0.87) or good (OR, 0.22; 95% CI: 0.07 a 0.62) compared to poor knowlegde. In addition, 4.56% (15/329) (95% CI: 2.57 to 7.41%) of veterinarians reported that they had brucellosis, of which 46.67% (7/15) considered that the disease was due to accidental exposure to anti- *B. abortus* live-attenuated vaccines. The prevalence of accidental exposure to anti-*Brucella* vaccine among veterinarians from Minas Gerais enrolled in the PNCEBT was high. The risk factors observed to unintentional contact with S19 and RB51 vaccine strains were the score of knowledge about human brucellosis symptoms and score of PPE use.

Keywords: *Brucella*, “job-related”, human brucellosis, vaccine, RB51, S19

Introduction

Brucellosis is one of the commonest bacterial zoonosis worldwide associated with reproductive failure in domestic animals and debilitating febrile illness in humans (Corbel et al., 2006; Pappas et al., 2006). Despite the great *Brucella* genus diversity of species , the majority of human infections are caused by *Brucella melitensis* and *Brucella abortus* (Franco et al., 2007). The disease has great impact on public health, since it is a zoonosis of strong occupational character (McDermott and Arimi, 2002), associated with chronic debilitating infection and high treatment costs (McDermott et al., 2013). Cattle farmers, slaughterers workers, microbiologists, veterinarians and their assistants are often exposed to infected animals, contaminated biological materials or live attenuated anti- *Brucella* vaccines capable of transmitting the disease to humans (Corbel et al., 2006). Moreover, these professionals can also become infected by non-occupational transmission route through the ingestion of milk and derivatives not submitted to heat treatment (Young, 1995).

In Brazil, bovine brucellosis caused by *B. abortus* is endemic and present in all the states, whereas *B. melitensis* is considered exotic in the country (Poester et al., 2002). However, the seroprevalence of positive herds exhibits a heterogenous distribution across the country, ranging from 0.91% [95% confidence interval (CI); 0.30 - 2.11] in Santa Catarina state (Baumgarten et al., 2016) to 30.60% [95% (CI); 27.40 - 34.00] in Mato Grosso do Sul state (Leal Filho et al., 2016). In order to reduce the brucellosis prevalence in cattle, the Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal – PNCEBT (National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis) was created in 2001 (MAPA, 2001). The PNCEBT is mainly based on compulsory vaccination of young females aged between 3 and 8 months with S19 and vaccination of females not vaccinated in this interval with RB51 (Dorneles et al., 2017), besides animal transit control, and slaughter of positive animals (Neto et al., 2016).

Since S19 and RB51 are live anti- *B. abortus* vaccines which, although effective and fundamental in the control of bovine brucellosis, are both pathogenic to humans (Joffe and Diamond, 1966; Nicoletti et al., 1986; Ashford et al., 2004), vaccination against brucellosis in Brazil must be performed only by veterinarians registered in the PNCEBT, or optionally by vaccinators registered under their responsibility (MAPA, 2017). This implies that the veterinarians and their assistants are among the most susceptible occupational group to human brucellosis, as besides dealing directly with sick animals, products of abortion and to perform deliveries, they are also exposed to live attenuated anti-*Brucella* vaccines (Proch et al., 2018). In fact, the risk of contracting brucellosis among professionals exposed to vaccine antigens was shown to be 5.40 times higher [95% (CI), 3.16 to 9.30] in comparison to professionals who were never exposed to anti-*Brucella* strains (Kutlu et al., 2014).

Among the pioneers states in the control of brucellosis in Brazil, Minas Gerais state have been enforcing compulsory vaccination of cattle and buffaloes young females in all its territory since 1994 (IMA, 1993). Nonetheless, although this strategy has led a significant reduction in the prevalence of seropositive herds in the comparative studies on the epidemiological situation of bovine brucellosis in Minas Gerais carried out 10 years apart (2002 – 2011) (Neto et al., 2016), the disease is still prevalent in the cattle herd with different ratios among producing regions, ranging from 2.02% [95% (CI), 0.41 to 3.62] in Leste to 5.06% [95% (CI), 2.56 to 7.56] in Triângulo Mineiro (Gonçalves et al., 2009; Oliveira et al., 2016).

In 2018, approximately 1.70 million bovine females were vaccinated in Minas Gerais state. In this context, accidental exposures to S19 and RB51 are highly prone to occur. Based on an estimate of 4 involuntary needle stick injuries per thousand inoculations among health professionals in North American hospitals (Henderson et al., 1990), it can be supposed about 6.80 thousand accidental inoculations with anti-*Brucella* vaccines in veterinarians in 2018 in Minas Gerais. However, since the conditions for handling and perform the vaccination in cattle are more adverse than the hospital ones (Ashford et al., 2004), this prediction is probably underestimated. Despite this, the incidence of human brucellosis in the state is unknown, since the legislation implementing its compulsory notification in the state of Minas Gerais only came into force from December 2018 (SES, 2018). Thereby, little is known about the epidemiological situation of occupational brucellosis among veterinarians and their assistants. Thus, the aims of this present study were estimate the prevalence of accidental exposure to S19 and RB51 vaccines and occupational brucellosis among veterinarians registered in the PNCEBT in Minas Gerais, as well as to identify the risk factors associated with accidental exposure to anti-*B. abortus* vaccines and understand the main perceptions and behaviors related to occupational brucellosis.

Materials and methods

Study design and area

This cross-sectional study was conducted from November 2018 to May 2019 in Minas Gerais state, located in southeastern Brazil with an area of 588,383 km^2. The state was divided in seven regions (strata) of bovine production as previously proposed in the epidemiological studies conducted in cattle (Figure 1) (Gonçalves et al., 2009; Oliveira et al., 2016) and validated by Alves et al., (2018). Each stratum exhibit different regional characteristics related to livestock activities, such as production systems, average herd size and sanitary practices adopted (Gonçalves et al., 2009; Oliveira et al., 2016; Alves et al., 2018).

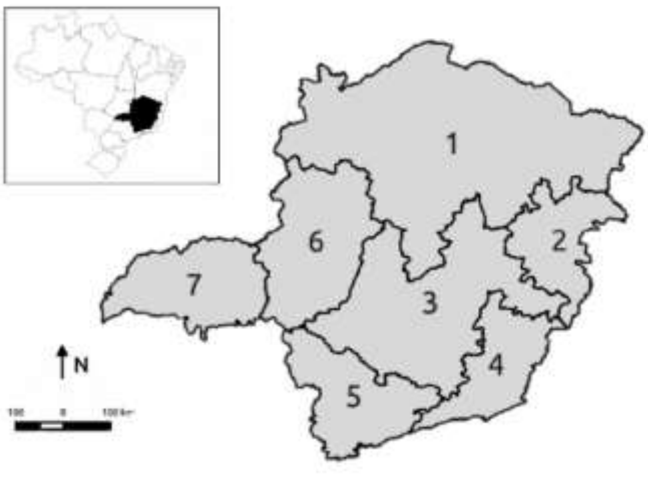


Figure 1: Map of the state of Minas Gerais, showing the regions defined in the current study. The state was divided into seven regions: 1. Noroeste, Norte and Nordeste; 2. Leste; 3. Central; 4. Zona da Mata; 5. Sul and Sudoeste; 6. Alto Paranaíba; and 7. Triângulo Mineiro (Oliveira et al., 2016).

Study population and eligibility criteria

The inclusion criteria comprised all veterinarians residing in Minas Gerais, enrolled in PNCEBT to perform brucellosis vaccination and who were actively vaccinating calves from January to June of 2018. On the other hand, the exclusion criteria removed all the professionals that had their contact information (email address) outdated in the register of veterinarians able to perform brucellosis vaccination from the Instituto Mineiro de Agropecuária – IMA (Agricultural Institute of Minas Gerais).

Sample size

The sample size was calculated using the following formula (Israel, 1992):

$$n_0 = \frac{Z^2 * p * q}{e^2} \qquad n_0 = \frac{(1.96)^2 * 0,5 * (1 - 0.5)}{0.05^2} \qquad n_0 = 384.16$$

where n_0 is the minimum sample size required, "Z" is the critical value for a given desired confidence level, "p" is the estimated proportion of the event to be studied, "q" is "1-p" and e is the desired level of precision. Since the estimated proportion of brucellosis prevalence among the study population was not known, p = 0.5 was assumed in order to obtain the largest sample size, with maximum variability. The desired confidence level was 0.95 and the precision 0.05. The study population was considered finite ($n \leq 0.05$), where N is the population number of individuals:

$$n = n_0/N \qquad n = 384.16/2{,}154 \qquad n = 0.18$$

The sample was then correct through finite population correction formula:

$$n = \frac{n_0}{1 + \frac{n_0}{N}} \qquad n = \frac{384.16}{1 + \frac{384.16}{2{,}154}} \qquad n = 326.02$$

The veterinarians were assigned proportionally to each different cattle producing regions in Minas Gerais state according to their residential address in the IMA's record. After obtaining the desired sample size, stratified sampling techniques were applied to calculate sampling population in each stratum. Then sample was drawn proportionally from each category by systematic random sampling to obtain the final sample size, always rounding off the decimal values to subsequent integer (Table 1) (Neyman, 1934). The number of veterinarians randomly selected from IMA's register by means of a draw, was four times greater (proportional per stratum) than the final sample size calculated, considering a 25% response rate. A total of 1,316 veterinarians were contacted by e-mail with the invitation for participation, informed consent term and questionnaire link. The emails were sent to the participants remembering then about the questionnaire until the minimum required sample size was reached for each stratum, totalizing 329 respondents (surplus responses were ruled out by saturation).

Table 1: Distribution of veterinarians residing in Minas Gerais, registered to perform brucellosis vaccination and who were actively vaccinating calves from January to June of 2018, according to bovine productive regions.

Strata	N	Relative frequency (%)	n*
Noroeste, Norte and Nordeste	200	9.29	31
Leste	116	5.39	18
Central	647	30.04	98
Zona da Mata	252	11.70	39
Sul and Sudoeste	462	21.45	70
Alto Paranaíba	220	10.21	34
Triângulo Mineiro	257	11.93	39
Total	2154	100.00	329

N = population; n= sample number * always rounded up

Ethical considerations

This study was approved by Human Ethics Research Committee (Comitê de Ética em Pesquisa com Seres Humanos) from Universidade Federal de Lavras (UFLA) (86861018.2.0000.5148). Informed consent term was obtained from all the participants before questionnaire administration.

Questionnaire survey

Data was obtained through an online questionnaire (S1 and S2 Appendix) based on a similar study conducted in Turkey (Kutlu et al., 2014), with some modifications. The questionnaire, translated from the original English version into the local language (Portuguese), was pre-tested by means of a pilot study with 20 veterinarians, in order to lead improvements in the data collection instrument. To prevent ‘leading line questioning’, general questions (containing closed, semi-closed and open questions), such as age, job experience, area of expertise, disease perceptions, infection control practices and risky procedures (vaccine administration and veterinary care related to bovine reproduction) were asked first. Then, specific questions related to occupational *B. abortus* infection and accidental S19 and RB51 unprotected contact were requested. Those individuals who reported brucellosis or unintentional exposition to live attenuated anti- *Brucella* vaccines were asked about the probable causes of the outcome, type of exposure to the S19 and RB51 strains, prophylaxis measures adopted, diagnostic methods used, symptoms occurrence and duration, treatments implemented and possible relapses of the disease.

Outcome definitions

B. abortus infection and accidental anti-*Brucella* vaccine exposure were based on the self-report of the participant.

Descriptive analysis

After reach the minimum required sample size for each stratum, all responses were imported into R statistical software 3.5.2 (Team, 2018), cleaned and checked for duplicates. To perform the analysis, participants data had to include at least the 28 required questions (S3 Appendix). Descriptive statistics of the variables were examined, frequency distributions for categorical variables and median, average, interquartile range and standard deviation values for continuous variables were calculated (S3 Appendix).

Transformations of variables

In order to assess the knowledge about brucellosis and prevention measures adopted by the respondents, three re-categorizations were performed in the variables. Variables concerned about the use of gloves, coat, protection goggles and masks (X13 to X16 – S3 Appendix) were grouped into a single variable: personal protective equipment (PPE) use. For this, scores were awarded for both the equipment, according to their importance in the prevention of the disease, and for the frequency of use. The variables gloves, mask, goggles and coat were re-categorized using, respectively, weight 4, 4, 2 and 1, and the weights of the frequencies of use were 2 for always, 1 for sometimes and 0 for none. Then, the sum of the values of each equipment multiplied by the respective frequency of use was performed, generating values between 0 (never used any protection) and 20 (always used all the PPE).

To identified the knowledge of the participants on brucellosis transmission (X22 – S3 Appendix), it was established a score, considering the follow decreasing importance order: transmission routes related to occupational risks (“self-inoculation with vaccines S19 and RB51” and “unprotected contact with products of potentially contaminated abortions” – weight = 2), infection sources not related to labor activities (“ingestion of milk and derivatives not submitted to thermal treatment” – weight = 1) and wrong answers about the disease spread (“direct contact with saliva of bovine/buffalo patients” and “ingestion of undercooked meat” – weight = 0). Then, the number of points of each participant were summed and the brucellosis transmission knowledge were scored as good (4 or 5 points), average (1, 2 or 3 points) and poor (0 points).

Lastly, the knowledge of the participants about the main symptoms of human brucellosis (X23 – S3 Appendix) were also evaluated. A similar principle was used, attributing scores to the alternatives based on the frequency of symptoms most related to clinical manifestation of *B. abortus* human infection. The values assigned for each alternative were 2 for “pain in the joints, sweating, fever and chills”, 1 for “endocarditis and orchitis can occur in severe cases” and 0 for “mainly reproductive clinical signs, as well as for bovine/buffaloes” and “staggering walking and mental disorientation in the first day after infection”. Then, the number of points of each participant were summed and the knowledge about brucellosis main symptoms were scored in good (2 or 3 points), average (1 points) and poor (0 points).

Statistical analysis

Apparent prevalence of accidentally vaccine exposure and occupational brucellosis was calculated by dividing the number of self-reported outcomes by the total number of veterinarians sampled. The CIs of these prevalences were obtained by the exact binomial distribution. A model for accidental exposure to anti-*Brucella* vaccines were fitted. The independent variables for the model are summarized in the S3 Appendix. The model was built using purposeful selection of variables in logistic regression according to Hosmer et al. (2013).

Briefly, preliminary analyses were carried out for each one of the variables considered as potential predictor variables. Chi-square test (χ^2) or Fisher exact test was performed for the qualitative variables and univariable logistic regression model was carried out for the quantitative ones. Variables that univariable test had a p-value less than 0.25 were considered as a possible candidate for the first multivariable model. Then, as the second step, a multivariable logistic model was fitted containing all the variables that reached the inclusion. The importance of each of them was assessed through the Wald test, variables that had a p-value greater than 0.05 were removed and a new model was fitted. The partial likelihood ratio test was used to compare the new and more parsimonious model to the old one. In the third step, it was observed if there was a variation greater than 20% in each one of coefficients when the variable was excluded from the model. In this case, the variable was added back into the model. The second and third steps were repeated for all non-significant variables. Following, all the variables not selected in the first step were added in the model one at a time. The fifth step consisted in verifying if the logit of each continuous variable of the model showed a linear relation as a function of the covariate. Then, the interactions of the variables were checked and adjusted, in the sixth step. The goodness-of-fit of the full model was assessed in the seventh step by the Receiver Operating Characteristic (ROC) curve and the Hosmer and Lemeshow test. The association among the dependent and independent variables in the final logistic regression model was calculated by odds ratios (OR) and their respective 95% CI.

All the statistical analysis were performed in R statistical software 3.5.2 (Team, 2018).

Results

Descriptive analysis

A total of 418 veterinarians ansewered the questionnaire. However, only 329 were included in the analysis due the stratification methodology proposed in the study (Table 1). Of the 329 participants, 273 (82.98%) were male and 56 (17.02%) female. The average age and professional experience were 40.63 (± 12.13) and 14.49 (± 11.65) years, respectively. The main fields of work reported were dairy cattle (65.05%) and beef cattle (17.93%), followed by others (17.02%). Moreover, 71.43% of the veterinarians were self-employed, 20.36% worked in a private company and 8.21% were public servers. The knowledge about bovine brucellosis transmission was considered good for 47.42%, mean for 51.98% and poor for 0.61% of the participants, while the knowledge about human brucellosis symptoms was evaluated as good for 83.59%, mean for 11.25% and poor for 5.17% of the respondents.

When asked about the number of procedures performed in the last six months, 93.92% reported vaccination against bovine brucellosis, 72.95% performed parturition assistance, 54.41% manual placenta removal and 53.80% had contact with abortion. The adherence to use of gloves, coat, goggles and coat during these procedures are shown in Figure 2. Among the reasons for not using the PPE, the most cited was lack of habit (46.71%), difficulty performing the procedure using protection (27.85%), not having the equipment (12.91%), not considering it important (10.25%) and lack of time (10.25%). The main forms of S19 and RB51 disposal cited were: infectious waste (31.61%), general waste from rural property (28.27%), urban general waste (11.55%), bury in the rural property (11.25%), burn (sometimes using the iron heater) (8.81%) and return to the agricultural store (8.51%).

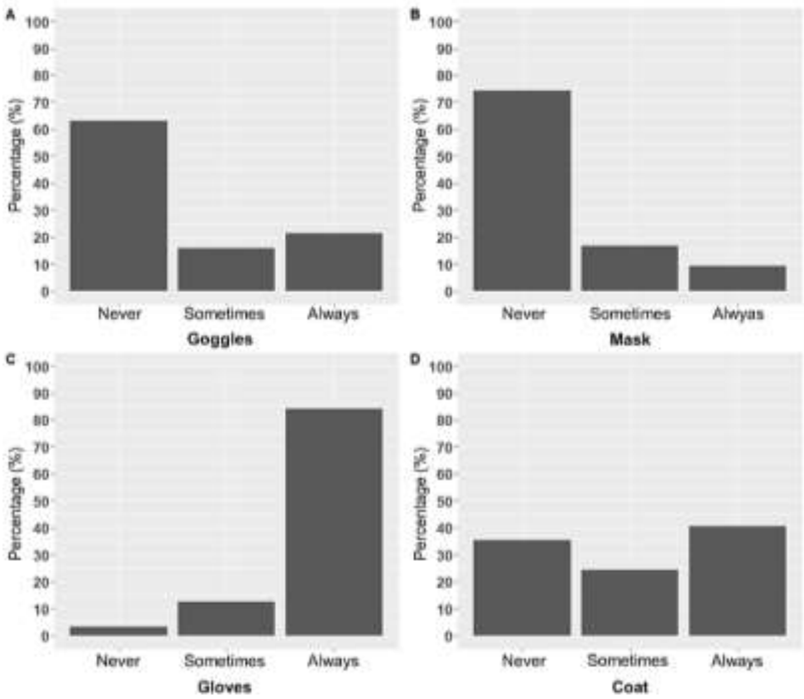


Figure 2: Frequency of use of personal protective equipment reported among veterinarians registered to perform bovine brucellosis vaccination in Minas Gerais, Brazil, 2018/2019: (a) goggles, (b) mask, (c) gloves and (d) coat.

About a quarter of the veterinarians sampled (25.84%) had a vaccinator registered under its responsibility and 95.29% reported having provided training to these professional to carry out the vaccination in animals. The knowledge of the veterinary about the use of PPE by the vaccinator showed that 64.71% reported not wearing mask, 52.94% coat, 51.76% goggles and 2.35% gloves. The vaccinators were exposed only to S19 strain, 7 (63.64%) once, 1 (9.09%) twice and 3 (27.27%) more than twice. Among these individuals, only 6 (54.55%) seek medical attention, 2 (18.18%) of them reported pain at the site of inoculation and fever, one (9.09%) associated with muscle pain and the other (9.09%) with weakness and headaches.

Nearly one-third of the veterinarians sampled, 32.83% (95% CI: 27.78 to 38.19%) reported having been accidentally exposed to S19 strain, 45.37% once, 27.78% twice and 23.15% more than twice, whereas RB51 exposure was reported by 2 individuals, once (1) and more than twice (1). Furthermore, 2 individuals described having been exposed to both vaccine strains. The forms of exposition reported were needlestick injury (37.74%), contact of not wounded skin with vaccine content (30.82%), splashing of vaccine content into eyes (20.75%) or into oral/nasal mucosa (6.29%), and contact of wounded skin with vaccine content (4.40%). The accident occurred mainly during the vaccine bottle manipulation (45.52%), followed by livestock vaccination (40.30%), disassembling the syringe (5.22%), re-capping the needle (5.22%) and disposal of materials (3.73%). Among the probable reasons for accidental exposition to S19 and RB51 strains were mentioned lack of infrastructure of the property to carry out the vaccination (34.68%), adoption of inadequate protection measures (26.61%), temperament of the animal (cattle breed) (17.74%), lack of attention or hurry (7.26%), pressure inside vaccine bottle when needle was inserted (4.84%), lack of knowledge about risks associated with brucellosis vaccination (1.61%) and other reasons (7.26%). Among the protection measures adopted during the accidental exposure, only 0.93% reported using all the PPE preconized, 48.15% were using only one PPE (coat, gloves, mask and goggles), 33.33% two, 4.63% three and 12.96% none. After unintentional contact with the anti-*Brucella* vaccine 47.09% washed the local, 32.56% performed the disinfection with an antiseptic, 11.05% sought medical attention, 4.07% performed self-medication, 1.74% conducted cauterization of the local and 3.49% did nothing. The drugs used in self-medication were doxycycline (28.57%), amoxicillin (14.29%), rifampicin + tetracycline (14.29%) and sulfonamide (14.29%), besides, curiously 28.57% reported using a veterinary spray based on tetracycline in the site of vaccine contact.

The prevalence of self-reported occupational brucellosis among the veterinarians sampled was 4.56% (15/329) (95% CI: 2.57 to 7.41%). Among these individuals 46.67% considered that brucellosis was due to accidental exposure to S19 and RB51, while 40.00% attributed the disease to unprotected contact with uterine secretions from infected animals, 6.67% to ingestion of raw milk or unprotected contact with uterine secretions from infected animals during a surgical procedure and 6.67% did not known the probable source of the *B. abortus* infection. The most frequent clinical signs reported were muscle and joint pain (46.67%), weakness, chills and sweating (33.33%), fever (26.67%), headaches (20.00%), weight loss (13.33%) and diarrhea (6.67%). Two individuals who had brucellosis described relapses with joint involvement (1) and skin allergy (1). The diagnostic methods used, the seek for medical care and therapeutic protocols implemented among the participants that self-declared *B. abortus* infection are shown in Table 2.

Table 2: Diagnostic methods used, the seek for medical care and therapeutic protocols implemented among veterinarians from Minas Gerais, Brazil (2018/2019) who reported *B. abortus* infection.

Patient	**Probable source of *B. abortus* infection**	**Seek medical**	**Diagnostic methods***				**Therapeutic protocol**
			iELISA	**PCR**	**RBT**	**Culture**	
1	Unprotected contact with uterine secretions from infected animals	Yes	Positive	-	Positive	-	Penicillin or cephalosporin (8 to 14 days) and Aminoglycoside (8 to 14 days)
2	Accidental exposure to S19 and RB51 strains	No	-	-	-	-	-
3	Unprotected contact with uterine secretions from infected animals	Yes	Positive	Positive	Positive	-	Doxycycline (more than 30 days) and rifampicin (22 to 30 days)
4	Unprotected contact with uterine secretions from infected animals	No	Positive	-	-	-	-
5	Accidental exposure to S19 and RB51 strains	Yes	Positive	-	Negative	-	Doxycycline (22 to 30 days) and rifampicin (22 - 30 days)
6	Unprotected contact with uterine secretions from infected animals	No	Positive	-	-	-	-
7	Accidental exposure to S19 and RB51 strains	Yes	-	-	-	Positive	Doxycycline (more than 30 days) and rifampicin (15 to 21 days) and Trimethoprim + Sulfamethoxazole (more than 30 days)
8	Unprotected contact with uterine secretions from infected animals during a surgical procedure and ingestion of unpasteurized milk	Yes	-	-	Positive	-	-
9	Not known	No	Positive	-	-	Negative	Doxycycline (more than 30 days) and Rifampicin (more than 30 days)
10	Unprotected contact with uterine secretions from infected animals	No	-	-	-	-	-
11	Accidental exposure to S19 and RB51 strains	Yes	Positive	-	-	-	Doxycycline (more than 30 days) and Rifampicin (more than 30 days) and Aminoglycoside (1 to 7 days)
12	Unprotected contact with uterine secretions from infected animals	No	-	-	Positive	-	-
13	Accidental exposure to S19 and RB51 strains	Yes	-	-	Positive	-	Other Tetracycline (15 to 21 days)
14	Accidental exposure to S19 and RB51 strains	Yes	Positive	-	-	-	Doxycycline (more than 30 days)
15	Accidental exposure to S19 and RB51 strains	No	Positive	-	-	-	Aminoglycoside (22 to 30 days)

**Brucella* standard agglutination test, 2-Mercaptoethanol and Coombs test were not performed; “-“ = Not performed; iELISA = Indiretc ELISA; RBT = Rose bengal plate test

Logistic regression model:

Variables that exhibited p-values lower than 0.25 in the univariate analysis and, thereby were included in the first multivariate logistic regression analysis of potential risk factors for accidental anti-*Brucella* vaccine exposure are summarized in Table 3.

Table 3: Results of univariate analysis for accidental anti-*Brucella* vaccine exposure among veterinarians from Minas Gerais, Brazil, 2018/2019.

Variable	Method	p Value
Year of birth	Fisher's exact test	0.25
Gender	Pearson's chi-squared test	0.11
Using gloves during work	Fisher's exact test	0.04
Using coat during work	Pearson's chi-squared test	0.13
Using mask during work	Pearson's chi-squared test	0.07
Score of knowledge (brucellosis symptoms)	Pearson's chi-squared test	0.01
Score of PPE use during work	Univariate logistic regression	0.01

PPE = Personal protective equipment

The final multivariate logistic model for this outcomes is shown in Table 4. The variables score of knowledge about brucellosis symptoms and score of PPE used during labor activities were significant for accidental S19 and RB51 accidental exposure and included in the model.

Table 4: Multivariate analysis for accidental anti-*Brucella* vaccine exposure among veterinarians from Minas Gerais, Brazil, 2018/2019.

Variable	OR	95% CI	p Value
Score of knowledge about brucellosis symptoms:			
Poor	-	-	-
Average	0,26	0,07 a 0,87	0,03*
Good	0,22	0,07 a 0,62	0,01*
Score of PPE use during work:			
Increase of one point	0,94	0,89 a 0,98	0,01*

*$p \leq 0.05$; OR = Odds ratio; CI = Confidence interval; PPE = Personal protective equipment

Discussion

Veterinarians are one of the most important risk group for occupational brucellosis because of the high exposure to infected animals, their contaminated fluids and to live attenuated anti-*Brucella* vaccines. Hence, this study aimed to identify the epidemiological situation of accidental exposure to S19 and

RB51, besides to characterize the practices and perception of veterinarians related to occupational brucellosis in Minas Gerais, Brazil. It was observed that the adoption of individual protection measures, as well as a good knowledge about the disease are important factors in the prevention of the occupational exposition to this zoonosis. Such results are crucial to direct public health policies aimed at worker health surveillance and strategic actions based on continuing education and awareness of veterinarians, mainly about the risks and characteristics of brucellosis as an occupational disease.

A great difficulty in researches conducted by means of online questionnaires is the low adherence of participants, in order to minimize this issue, an adhesion rate of 25% was considered. This rate was adopted after the application of the pilot questionnaire (data not shown) and it is corroborated by a study involving British veterinarians, in which even with a forecast of 30% of adhesion, the minimum number of participants required was not reached (Robin et al., 2017). On the other hand, the use of online platforms makes the epidemiological surveys cheaper, faster and affordable, compared to classical methodologies.

The higher proportion of males observed among participants can be justified by the profile of the occupation described in the country 15 years ago, the average job experience of the participants, which showed a predominance of male veterinarians among those working with large animals (CFMV, 1999). In this field, veterinary services related to the reproductive system, including parturition assistance, removal of placenta and abortions care, as well as vaccination of animals against brucellosis, are frequent, which implies to professionals working with dairy and beef cattle have greater probability of contact with *B. abortus.* Since unprotected contacts with animal biological fluids and live attenuated vaccines poses a great risk for occupational brucellosis, protection measures adopted by the participants, PPE adherence and barriers to the non-use of PPE were questioned. More than half of the participants reported never wearing mask or goggles, two equipment considered very important for human brucellosis prevention and that were scored with high weights in the present study. Moreover, it is alarming to observe that an expressive proportion of the participants (6.19% for goggles and

10.59% for mask) did not consider important the use these PPEs and that 12.46% (41/329) were classified as having an insufficient knowledge about the disease transmission to humans, not considering as true the following alternatives: "self-inoculation with vaccines S19 and RB51" and "unprotected contact with potentially contaminated abortions". Likewise, poor understanding of main clinical signs of human brucellosis was identified and is discussed further as a probable cause of the low prevalence of self-reported occupational brucellosis.

Additionally, inadequate disposal of the anti-*Brucella* vaccines and accidental exposure to S19 and RB51 among vaccinators registered under veterinarians' responsibility were also identified. Proper disposal of biological products at the farm is as important as proper use (Gunn et al., 2013). Vaccine bottles and vaccination residues disposed at the property in rural general waste or buried are a source of infection to other animals, domestics and wild, as well as can contaminate the environment (soil and water). Similarly, disposal of vaccination residues in urban waste is inappropriate and can lead to infection in workers from disposal companies not prepared to receive infectious products (Compés Dea et al., 2017). Another improper form of disposal reported by the respondents was incineration, since the vaccine bottle can burst and spread aerosols, a common route of infection for human brucellosis (Kaufmann et al., 1980).

Concerning to vaccine accidents among veterinary assistants, most of the veterinarians reported that their assistants did not wear a mask, coat or goggles during occupational activities, although they affirmed to have trained these assistants to handle the anti-*Brucella* vaccines. Incident among vaccinators who carry out vaccination under veterinarian's responsibility have been informed by 12.94% (11/85) participants, all to S19, which is almost three times lower compared to unintentional contact with vaccine among veterinarians [108/329 (32.83%)]. These results were different from those observed by Proch et al. (2018), that found more occupational brucellosis among assistants than among veterinarians, probably because our study did not performed laboratorial tests, being the outcome reported by the veterinarian and not by the technician, which could lead to an underreported number.

For this reason, information about the main causes and consequences of brucellosis accidental exposure in veterinarians could be considered more precise compared to data on vaccinators.

The prevalence of accidental exposure to anti-*Brucella* vaccines (32.83%) observed in the present study was almost two times higher than that reported on veterinary and veterinary assistants in 2011 in Turkey (17.34%). This information is alarming due the great participation of vaccine exposure in brucellosis related to labor activities among veterinarians in Minas Gerais. This large number of reported vaccine accidents is probably associated with insufficient farm infrastructure to safely perform the vaccination, which can be explained by the occurance of these problems especially in small properties, very common in the state of Minas Gerais, where the structure of the facilities are usually deficient (Gonçalves et al., 2009; Oliveira et al., 2016; Alves et al., 2018). Moreover, Minas Gerais has the third cattle herd of the country (IBGE, 2017) and perform around 1.70 million of brucellosis vaccinations per year , which promotes more opportunities for involuntary exposition to vaccines.

For both veterinarians and veterinary assistants, exposure to S19 was more frequent than to RB51, which was expected since in Brazil, vaccination of young heifers against brucellosis is compulsory and habitually performed with S19, being RB51 most commonly used when animals exceed 8 months of age without being properly immunized (MAPA, 2017). Moreover, S19 usually costs a quarter of the value of RB51 and is more easily found in the market, which also contribute to its use. In accordance to previously published studies, more than half of the participants reported needle-stick injury as the main form of accidental exposure to the anti-*Brucella* vaccine (Joffe and Diamond, 1966; Nicoletti et al., 1986; Blasco and Díaz, 1993; Ashford et al., 2004; Kutlu et al., 2014; Proch et al., 2018), which happened mainly during the handling of the vaccine bottle or during vaccination. It may have also contributed to cause the involuntary contact with the vaccine the low infrastructure of the property, attributed as the cause of the accident by most of the respondents, followed by inappropriate use of protective measures and animal's temperament. In fact, vaccine bottle manipulation, disassembling the syringe, re-capping the needle and disposing of materials are frequent causes of reported accidents among health professionals (Cullen et al., 2006; Weese and Jack, 2008; Fowler et al., 2016) and most professionals reported using only one, two or none PPE during the accidental contact with anti-*Brucella* vaccine strains. Furthermore, a study carried out in Italy also demonstrated that three-fourth of needle-stick injuries were due to incorrect needle handling by health care workers, and that one third could be avoided by the use of safety devices (Castella et al., 2003). However, exposure through aerosol in the oral/nasal mucosa and into eyes should be highlighted as another possible way of contact with *B. abortus* vaccines due to the low adherence to the use of mask and goggles observed. Additionally, it should be noted that associated to low rates of demand for medical care, inadvisable practices were described by some of the participants, including self-medication, use of veterinary drugs or even skin cauterization. These practices, besides inadequate, can be invasive and dangerous.

The prevalence of self-reported brucellosis (4.56%) observed was lower compared to the prevalence (11.8%) found by Kutlu et al. (2014) among veterinarians and veterinary assistants in Turkey. Nonetheless, unlike Brazil, Turkey is endemic for both *B. abortus* and *B. melitensis*, being the last responsible for more severe clinical signs in humans (Franco et al., 2007), which could lead to a better perception of the participants about the occurrence of the disease. Indeed, human brucellosis caused by *B. melitensis* tends to be less underdiagnosed than brucellosis caused by *B. abortus* (Pappas et al., 2005). Moreover, as the field strain, *B. melitensis* vaccine strain REV-1 is more virulent than *B. abortus* vaccine strains (S19 and RB51), which could explain the higher (60.71%) percentage of Turkish professionals infected by *Brucella* spp. after unprotected contact with vaccine compared to the results of the present study (46.67%). As expected, followed by vaccine accident, the second major cause attributed to occupational brucellosis was unprotected contact with uterine secretions from sick animals. In addition, even the individual that credited his infection to the consumption of raw milk also considered the possibility of becoming infected due to unprotected contact with animal fluids during a surgical procedure. Also, in agreement with the literature, the most frequently reported clinical signs reported were joint involvement, weakness, fever, chills and headaches (Young, 1995). However, it is worth to mention that an important deficiency was identified regard the knowledge of the veterinarians about the clinical signs of human brucellosis, since almost a quarter of the veterinarians chosen, among other alternatives, the "clinical signs mainly reproductive as well as for bovine / buffaloes as an option for human brucellosis symptoms”, which may have resulted in a possible underreported number of outcomes. Indeed, the self-report of brucellosis is impaired if the individual does not know the symptoms of the disease in man. Furthermore, the results revealed a predominance of indirect methods in the diagnosis of the disease, as well as observed in a systematic review and meta-analysis on occupational brucellosis (Pereira et al., Unpublish data), which could by justified considering the lower risks and costs involved in indirect methods compared with bacterial isolation and molecular techniques. Other results that also deserves especial attention are the seek for medical attention and the treatment received, since even around fifty percent of the veterinarians who reported brucellosis have sought for specialized medical care, some have been inadequately treated for the disease (Table 2). We must consider that infections caused by facultative intracellular bacteria as *Brucella* are often chronic (Gorvel and Moreno, 2002), and thereby a minimum period of four to six weeks of treatment with a combination of drugs is recommended, in order to avoid relapses (Yousefi-Nooraie et al., 2012; SES, 2015). The most commonly used and recommended regimens are those that combine doxycycline and an aminoglycoside or rifampicin (Ariza et al., 2007). Two cases of relapses were reported even using a prolonged treatment and a combination of drugs; however, it was not possible to identify whether the protocol reported occurred before or after the resurgence of the clinical signs. These results evidence the lack of knowledge and the unprepared of many health professionals to deal with the disease, contributing to the neglected situation of human brucellosis.

In the multivariate logistic regression model for vaccine accidental exposure, it was observed that at each point increased in the PPE use score, the individuals were 0.94 times more likely (95% CI: 0.89 to 0.98) to not been accidentally exposed to S19 or RB51 strains compared to individuals in the immediately lower score. In fact, PPE limits human exposure to infectious sources: gloves and coat provides a skin protection against vaccines splashing and mask and goggles prevent brucellosis airborne and conjunctival transmission (Cash-Goldwasser et al., 2018). Also, individuals with poor knowledge about human brucellosis symptoms were more likely to had an accidental exposure to anti-*Brucella* vaccines when compared to veterinarians with mean (OR, 0.26; 95% CI: 0.07 to 0.87) or good (OR 0.22; 95% CI: 0.07 to 0.62) knowledge. This is probably because exposure to the vaccine is directly related to perception of the professional about the risk: the higher the knowledge, the lower the chances of exposing themselves to danger.

A model of risk factors for occupational brucellosis were build (data not shown). However, it was not possible to reach a robust model with a satisfactory fit, probably due to the fact that the outcome of occupational brucellosis was identified from a self-report by professionals. who demonstrated to have insufficient knowledge about the symptomatology of the disease. Since veterinarians have shown insufficient knowledge about brucellosis clinical signs, a possible perception bias may have contributed to the low prevalence of occupational brucellosis among this group and consequently a poor fit of the model.

The sampling carried out in the present study, among the veterinarians from Minas Gerais involved in the PNCEBT, allowed to draw a profile on the individuals who carries out routine vaccination against bovine brucellosis, and thereby, to know in detail how these professionals understand and deal with the inherent risks associated with vaccination and their work activities. In future studies, to estimate a more accurate prevalence of occupational brucellosis, it is interesting to perform a laboratory diagnostic among the individuals, in order to control the under diagnostic bias due self-report.

Conclusions

The prevalence of accidental exposure to anti-*Brucella* vaccine among veterinarians from Minas Gerais enrolled in the PNCEBT was high and the risk factors observed to unintentional contact with S19 and RB51 vaccine strains were the score of knowledge about human brucellosis symptoms and score of PPE use.

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References

Alves, C.d.M., Dorneles, E.M.S., Oliveira, L.F.d., Neto, J., Gonçalves, V.S.P., Lôbo, J.R., Heinemann, M., Lage, A.P., 2018. Productive profile of cattle-rearing farms in the state of Minas Gerais, Brazil, 2002.

Ariza, J., Bosilkovski, M., Cascio, A., Colmenero, J.D., Corbel, M.J., Falagas, M.E., Memish, Z.A., Roushan, M.R.H., Rubinstein, E., Sipsas, N.V., Solera, J., Young, E.J., Pappas, G., International Society of, C., Institute of Continuing Medical Education of, I., 2007. Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. PLoS Med 4, e317-e317.

Ashford, D.A., di Pietra, J., Lingappa, J., Woods, C., Noll, H., Neville, B., Weyant, R., Bragg, S.L., Spiegel, R.A., Tappero, J., Perkins, B.A., 2004. Adverse events in humans associated with accidental exposure to the livestock brucellosis vaccine RB51. Vaccine 22, 3435-3439.

Baumgarten, K.D., Veloso, F.P., Grisi-Filho, J.H.H., Ferreira, F., Amaku, M., Dias, R.A., Telles, E.O., Heinemann, M.B., Gonçalves, V., Ferreira Neto, J.S., 2016. Prevalence and risk factors for bovine brucellosis in the State of Santa Catarina, Brazil. Semina: Ciências Agrárias 37, 3425.

Blasco, J.M., Díaz, R., 1993. *Brucella melitensis* Rev-1 vaccine as a cause of human brucellosis. The Lancet 342, 805.

Cash-Goldwasser, S., Maze, M.J., Rubach, M.P., Biggs, H.M., Stoddard, R.A., Sharples, K.J., Halliday, J.E.B., Cleaveland, S., Shand, M.C., Mmbaga, B.T., Muiruri, C., Saganda, W., Lwezaula, B.F., Kazwala, R.R., Maro, V.P., Crump, J.A., 2018. Risk Factors for Human Brucellosis in Northern Tanzania. Am J Trop Med Hyg 98, 598-606.

Castella, A., Vallino, A., Argentero, P.A., Zotti, C.M., 2003. Preventability of percutaneous injuries in healthcare workers: a year-long survey in Italy. J Hosp Infect 55, 290-294.

CFMV, 1999. Contribuição para o delineamento do perfil do mercado de trabalho do médico veterinário e do zootecnista no Brasil. CFMV, Brasília - Distrito Federal.

Compés Dea, C., Guimbao Bescós, J., Alonso Pérez de Ágreda, J.P., Muñoz Álvaro, P.M., Blasco Martínez, J.M., Villuendas Usón, M.C., 2017. Epidemiological investigation of the first human brucellosis case in Spain due to *Brucella suis* biovar 1 strain 1330. Enfermedades Infecciosas y Microbiología Clínica 35, 179-181.

Corbel, M.J., Elberg, S.S., Cosivi, O., 2006. Brucellosis in humans and animals. World Health Organization.

Cullen, B.L., Genasi, F., Symington, I., Bagg, J., McCreaddie, M., Taylor, A., Henry, M., Hutchinson, S.J., Goldberg, D.J., 2006. Potential for reported needlestick injury prevention among healthcare workers through safety device usage and improvement of guideline adherence: expert panel assessment. J Hosp Infect 63, 445-451.

Dorneles, E.M.S., Oliveria, L.F., Lage, A.P., 2017. *Brucella abortus* Vaccines: Use in Control Programs and Immune Response. Journal of Bacteriology and Mycology 4, id1044.

Fowler, H.N., Holzbauer, S.M., Smith, K.E., Scheftel, J.M., 2016. Survey of occupational hazards in Minnesota veterinary practices in 2012. Journal of the American Veterinary Medical Association 248, 207-218.

Franco, M.P., Mulder, M., Gilman, R.H., Smits, H.L., 2007. Human brucellosis. The Lancet Infectious Diseases 7, 775-786.

Gonçalves, V.S.P., Delphino, M.K.V.C., Dias, R.A., Ferreira, F., Amaku, M., Ferreira Neto, J.S., Porto, T.B., Alves, C.M., Figueiredo, V.C.F., Lôbo, J.R., 2009. Situação epidemiológica da brucelose bovina no Estado de Minas Gerais. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 61, 35-45.

Gorvel, J.P., Moreno, E., 2002. Brucella intracellular life: from invasion to intracellular replication. Veterinary microbiology 90, 281-297.

Gunn, D., Jensen, K., Williams, S., Parsons, C., Hudson, T., England, J., 2013. Cattle vaccine handling and management guidelines. University of Idaho. PNW 637.

Henderson, D.K., Fahey, B.J., Willy, M., Schmitt, J.M., Carey, K., Koziol, D.E., Lane, H.C., Fedio, J., Saah, A.J., 1990. Risk for occupational transmission of human immunodeficiency virus type 1 (HIV-1) associated with clinical exposures. Annals of Internal Medicine 113, 740-746.

Hosmer, D.W., Lemeshow, S., Sturdivant, R.X., 2013. Applied Logistic Regression. Wiley.

IBGE, 2017. Produção da pecuária municipal.

IMA, 1993. Portaria nº 083/93, de 09 de novembro de 1993. In: Agropecuária, I.M.d. (Ed.), Belo Horizonte, Minas Gerais.

Israel, G.D., 1992. Determining Sample Size. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Joffe, B., Diamond, M., 1966. Brucellosis due to self-inoculation. Annals of Internal Medicine 65, 564-565.

Kaufmann, A.F., Fox, M.D., Boyce, J.M., Anderson, D.C., Potter, M.E., Martone, W.J., Patton, C.M., 1980. Airborne spread of brucellosis. Annals of the New York Academy of Sciences 353, 105-114.

Kutlu, M., Ergonul, O., Sayin-Kutlu, S., Guven, T., Ustun, C., Alp-Cavus, S., Ozturk, S.B., Acicbe, O., Akalin, S., Tekin, R., Tekin-Koruk, S., Demiroglu, Y.Z., Keskiner, R., Gönen, I., Sapmaz-

Karabag, S., Bosnak, V., Kazak, E., 2014. Risk factors for occupational brucellosis among veterinary personnel in Turkey. Preventive veterinary medicine 117, 52-58.

Leal Filho, M., Francisca Neves Bottene, I., Almeida Retumba Carneiro Monteiro, L., Oliveira Pellegrin, A., Salvador Picão Gonçalves, V., Ferreira, F., Dias, R., Amaku, M., Telles, E., Filho, J.H.H.G., Heinemann, M., Neto, J., 2016. Control of bovine brucellosis from 1998 to 2009 in the State of Mato Grosso do Sul, Brazil.

MAPA, 2001. Instrução Normativa n. 2 de 10 de janeiro de 2001: Institui o Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal. In: Ministério da Agricultura, P.e.A. (Ed.) Diário Oficial da União, Brasília, Distrito Federal, 5.

MAPA, 2017. Instrução normativa nº 10, de 3 de março de 2017. In: Ministério da Agricultura, P.e.A. (Ed.) Diário Oficial da União, Brasília, Distrito Federal, 4-8.

McDermott, J., Grace, D., Zinsstag, J., 2013. Economics of brucellosis impact and control in low-income countries. Scientific and Technical Review of the Office International des Epizooties 32, 249-261.

McDermott, J.J., Arimi, S.M., 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. Veterinary microbiology 90, 111-134.

Neto, J.S.F., Silveira, G.B.D., Silveira, Rosa, B.M., Rosa, Gonçalves, V.S.P., Grisi-Filho, J.H.H., Amaku, M., Dias, R.A., Ferreira, F., Heinemann, M.B., Telles, E.O., Lage, A.P., 2016. Analysis of 15 years of the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis, Brazil. Semina: Ciências Agrárias 37, 3385-3402.

Neyman, J., 1934. On the two different aspects of the representative method: the method of stratified sampling and the method of purposive selection. Journal of the Royal Statistical Society 97, 558-625.

Nicoletti, P., Ring, J., Boysen, B., Buczek, J., 1986. Illness in a veterinary student following accidental inoculation of Brucella abortus strain 19. Journal of American College Health 34, 236-237.

Oliveira, L.F., Dorneles, E.M.S., Mota, A.L.A.A., Gonçalves, V.S.P., Ferreira Neto, J.S., Ferreira, F., Dias, R.A., Telles, E.O., Grisi-Filho, J.H.H., Heinemann, M.B., Amaku, M., Lage, A.P., 2016. Seroprevalence and risk factors for bovine brucellosis in Minas Gerais state, Brazil. Semina: Ciências Agrárias, Londrina 37, 3449-3466.

Pappas, G., Akritidis, N., Bosilkovski, M., Tsianos, E., 2005. Brucellosis. The New England journal of medicine 352, 2325-2336.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., Tsianos, E.V., 2006. The new global map of human brucellosis. The Lancet Infectious Diseases 6, 91-99.

Pereira, C.R., Cotrim, J.V., Oliveira, L.F., Lage, A.P., Dorneles, E.M.S., Unpublish data. Occupational exposure to human brucellosis infection: a systematic review and meta-analysis.

Poester, F.P., Goncalves, V.S., Lage, A.P., 2002. Brucellosis in Brazil. Vet Microbiol 90, 55-62.

Proch, V., Singh, B.B., Schemann, K., Gill, J.P.S., Ward, M.P., Dhand, N.K., 2018. Risk factors for occupational *Brucella* infection in veterinary personnel in India. Transbound Emerg Dis 65, 791-798.

Robin, C., Bettridge, J., McMaster, F., 2017. Zoonotic disease risk perceptions in the British veterinary profession. Preventive veterinary medicine 136, 39-48.

SES, P.-. 2015. Protocolo de manejo clínico e vigilância em saúde para brucelose humana no Estado do Paraná. In: (SES), S.E.d.S. (Ed.), 70.

SES, M.G.-. 2018. Resolução n. 6.532, de 05 de dezembro 2018: Acrescenta doenças, agravos e eventos de saúde pública de interesse estadual à lista nacional de doenças de notificação compulsória e dá outras providências. In: (SES), S.d.E.d.S. (Ed.).

Team, R.D.C., 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Weese, J.S., Jack, D.C., 2008. Needlestick injuries in veterinary medicine. Can Vet J 49, 780-784.

Young, E.J., 1995. An overview of human brucellosis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 21, 283-289; quiz 290.

Yousefi-Nooraie, R., Mortazhejri, S., Mehrani, M., Sadeghipour, P., 2012. Antibiotics for treating human brucellosis.

S1 Appendix: Questionnaire

Evaluation of brucellosis and accidental exposure to S19 and RB51 vaccines occurrence among veterinarians registered in Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal – PNCEBT (National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis) in Minas Gerais state:

Subtitle: ( ) or ○ = it is possible to select only one answer alternative per question
[ ] or □ = it is possible to select one or more answer alternative per question

1. Informed consent term:

I - Identification of the project Title of the experimental work: Risk factors for occupational brucellosis among veterinarians in Minas Gerais, Brazil. Institution: Universidade Federal de Lavras. Researchers in charge: Elaine Maria Seles Dorneles and Carine Rodrigues Pereira. Telephone for contact: (35) 3829-1148 E-mails: elaine.dorneles@ ufla.br or rpcarine@gmail.com

II - Clarifications: The methodology of the project is based on the application of the questionnaire "Evaluation of the occurrence of brucellosis and accidental exposure to vaccines S19 and RB51 among veterinarians in Minas Gerais", via Google forms, sent to participants by e-mail and telephone, provided by the IMA together with the name and city of each professional. There are no predictable risks or discomforts for your participation in the survey, other than the time taken for approximately 20 minutes to complete the questionnaire. All information collected will be used only for statistical analysis and personal data will be kept strictly confidential. You will not incur any costs in participating in the study and may stop participating or withdraw your consent at any time, without having to justify, and will not suffer any loss. There is no economic value, receivable or payable, for your participation. You are guaranteed your right to further clarification about the study and its consequences, in short, everything you want to know before, during and after your participation.

III - Voluntary participation: Your participation in any type of research is voluntary. In case of doubt about your rights, write or call UFLA's Human Research Ethics Committee. Address - Campus Universitário da UFLA, Pró-reitoria de pesquisa, COEP mailbox 3037, telephone: (35) 3829-5182. I declare that I have read and understood all the procedures that will be carried out in this work. I also declare that I have been informed that I may withdraw at any time. I hereby agree to participate as a volunteer for the research project described above. A copy of this term will be emailed to you automatically.

( ) I accept

( ) I do not accept *since you do not agree to the terms, you can not continue participating in the survey: end of the questionnaire*

2. Email: ____________

3. In which city do you live?

( ) List of the 853 municipalities of Minas Gerais state

4. Year of birth:

( ) Year list from 1948 to 1996

5. Gender:

( ) Male

( ) Female

6. How long have you been working as veterinary (years)?

( ) Less than a year

( ) List from 1 to 47 years

( ) 48 year or over

7. Year in which you were registered as a vaccinator in PNCEBT:

( ) List of years from 2002 to 2018

8. In which regions of cattle production do you work?
[ ] Alto Paranaíba
[ ] Central
[ ] Leste
[ ] Noroeste, Norte e Nordeste
[ ] Sul e Sudeste
[ ] Triângulo Mineiro
[ ] Zona da Mata

9. In addition to being registered, are you also enabled for brucellosis and tuberculosis diagnosis?
( ) No
( ) Yes

10. You mainly work with:
( ) Dairy cattle/buffalo
( ) Beef cattle/buffalo
( ) Small ruminants
( ) Dogs and cats
( ) Horses
( ) Administrative services
( ) Inspection
( ) Animal sanitary defense
( ) Other: ___

11. Which is your employment relationship?
( ) Self employed
( ) Private company
( ) Public server

12. Signal the amount of procedures performed in cattle/buffaloes in the last six months:

	0	1 - 5	6 - 10	≥ 11
Delivery	( )	( )	( )	( )
Manual removal of retained placenta	( )	( )	( )	( )
S19 or RB51 vaccine administration	( )	( )	( )	( )
Intervention of abortion or preterm delivery	( )	( )	( )	( )

13. Which personal protective equipment did you use while performing those procedures?

	Always	Sometimes	Never
Gloves	( )	( )	( )
Coat	( )	( )	( )
Protective goggles	( )	( )	( )
Mask	( )	( )	( )

14. If some answer was sometimes or never, why the equipment was not used?

	Does not apply	Hinder the procedure	Lack of time	Lack of habit	I do not own the equipment	I do not think it matters
Gloves	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Coat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Protective goggles	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Mask	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

15. How the materials used for vaccination against brucellosis are disposal?
[ ] General waste from rural property
[ ] Urban general waste
[ ] Infectious waste
[ ] The materials used are returned to the agricultural store
[ ] Bury in the rural property

16. In your opinion, what are the main routes of brucellosis transmission?
[ ] Unprotect contact with contamined abortion products
[ ] Direct contact with saliva from sick cattle/buffaloes
[ ] Ingestion of unpasteurized milk or milk products
[ ] Auto-inoculation with S19 and RB51 vaccines
[ ] Ingestion of undercooked meat

17. In your opinion, brucellosis is a disease that causes in humans:
[ ] Clinical signs mainly reproductive, as well as in animals
[ ] Joint pain, sweating, fever and chills
[ ] Walking staggering and mental disorientation in the first days of infection
[ ] Endocarditis and orchitis may occur in severe cases

18. Did you eat unpasteurized milk or milk products in the last six months?
( ) No
( ) Yes

19. Do you have registered vaccinators under your responsibility?
( ) No *Go to the question 27*
( ) Yes *Go to the question 20*

20. Did the vaccinator receive some type of training to carry out cattle/buffalo vaccination with S19 and RB51?
( ) No
( ) Yes

21. Which of these equipment does the vaccinator use during the handling of the S19 and RB51 vaccines?

	Yes	No	Not known
Gloves	( )	( )	( )
Coat	( )	( )	( )
Protection goggles	( )	( )	( )
Mask	( )	( )	( )

22. Did the vaccinator have ever report any accidental exposure to S19 or RB51 vaccines?
( ) No *Go to the question 27*
( ) Yes *Go to the question 23*
( ) Not known *Go to the question 27*

23. To which vaccine was the vaccinator exposed? How many times?

	None	One time	Two times	More than two times
S19	( )	( )	( )	( )
RB51	( )	( )	( )	( )

24. Did the vaccinator seek medical attention after accidental exposure to S19 or RB51?
( ) No
( ) Yes
( ) Not known

25. Did the vaccinator show any symptoms after accidental exposure to S19 or RB51 vaccines?
( ) No *Go to the question 27*
( ) Yes *Go to the question 26*
( ) Not known *Go to the question 27*

26. Which of the following symptoms did the vaccinator present?

	Yes	No	Not known
Pain at the site of inoculation	( )	( )	( )
Weakness	( )	( )	( )
Muscle aches	( )	( )	( )
Joint pain	( )	( )	( )
Weight loss	( )	( )	( )
Headaches	( )	( )	( )
Fever	( )	( )	( )
Diarrhea	( )	( )	( )
Vomiting	( )	( )	( )
Chills and sweating	( )	( )	( )

27. Did you ever have brucellosis?
( ) No *Go to the question 29*
( ) Yes *Go to the question 28*

28. Was brucellosis caused by accidental exposure to S19 or RB51?
( ) No *Go to the question 45*
( ) Yes *Go to the question 30*

29. Have you ever been accidentally exposed to anti-*Brucella* vaccine?
( ) No *end of the questionnaire*
( ) Yes *Go to the question 30*

30. In which year occurred your first accidental exposure to S19 or RB51?
( ) 1994 or before
( ) List of years from 1995 to 2018

31. What type of exposure has occurred?
[ ] Penetration of needle
[ ] Splashing of vaccine content into eyes
[ ] Splashing of vaccine content into oral/nasal mucosa
[ ] Contact of vaccine content to not wounded skin
[ ] Contact of vaccine content to wounded skin
[ ] Other ____

32. To which vaccine have you been exposed to? How many times?

	None	One time	Two times	More than two times
S19	( )	( )	( )	( )
RB51	( )	( )	( )	( )

33. At what point did the accident occur?
[ ] Vaccine bottle manipulation
[ ] Livestock vaccination
[ ] Disassembling the syringe
[ ] Disposal of materials
[ ] When re-capping the needle
[ ] Other _____

34. Which personal protective equipment did you use when you were exposed to the anti-*Brucella* vaccine(s)?
[ ] Gloves
[ ] Mask
[ ] Coat
[ ] Protection goggles
[ ] None
[ ] Other ___

35. What do you think was the reason for accidental exposure to the anti-*Brucella* vaccine?
[ ] Lack of knowledge about vaccine risks
[ ] Lack of infrastructure of the property to carry out the vaccination
[ ] Animal's temperament
[ ] Inadequate protection measures
[ ] Other ___

36. What did you do after the exposure?
[ ] Washed the place
[ ] Disinfection of the site with antiseptic
[ ] Seek medical attention
[ ] Self-medication
[ ] Nothing
[ ] Other ___

37. In case of self-medication after exposure to the anti-*Brucella* vaccine, what was the drug and protocol used? If you have not self-medicated, write NA. ________

38. Which of the following symptoms did you have when you were exposed to the anti-*Brucella* vaccine?

	None	1 – 3 days	3 – 7 days	7 – 15 days	15- 30 days	More than 30 days
Pain at the site of inoculation	( )	( )	( )	( )	( )	( )
Weakness	( )	( )	( )	( )	( )	( )
Muscle aches	( )	( )	( )	( )	( )	( )
Joint pain	( )	( )	( )	( )	( )	( )
Weight loss	( )	( )	( )	( )	( )	( )
Headaches	( )	( )	( )	( )	( )	( )
Fever	( )	( )	( )	( )	( )	( )
Diarrhea	( )	( )	( )	( )	( )	( )
Vomiting	( )	( )	( )	( )	( )	( )
Chills and sweating	( )	( )	( )	( )	( )	( )

39. Did you seek medical attention because of the symptoms?
( ) No
( ) Yes

40. Have you ever been tested for brucellosis due to exposure to the anti-*Brucella* vaccine? What was the result?

	Did not realize	Positive	Negative	Inconclusive
indirect ELISA	( )	( )	( )	( )
PCR	( )	( )	( )	( )
STAT	( )	( )	( )	( )
Rose Bengal	( )	( )	( )	( )
Coombs	( )	( )	( )	( )
2-ME	( )	( )	( )	( )
Culture	( )	( )	( )	( )

41. Did you take antimicrobials for post-exposure treatment to the anti-*Brucella* vaccine?
( ) No *end of the questionnaire*
( ) Yes *Go to the question 42*

42. Which antimicrobials were used in the treatment?

	None	1 – 3 days	3 – 7 days	7 – 15 days	15- 30 days	More than 30 days
Penicillin or cephalosporin	( )	( )	( )	( )	( )	( )
Ampicillin	( )	( )	( )	( )	( )	( )
Amoxicillin + clavulanate	( )	( )	( )	( )	( )	( )
Macrolides such as azithromycin, clarithromycin, erythromycin	( )	( )	( )	( )	( )	( )
Quinolones such as ciprofloxacin, levofloxacin	( )	( )	( )	( )	( )	( )
Doxycycline	( )	( )	( )	( )	( )	( )
Other tetracyclines such as minocycline	( )	( )	( )	( )	( )	( )
Aminoglycosides such as streptomycin, gentamicin	( )	( )	( )	( )	( )	( )
Rifampicin	( )	( )	( )	( )	( )	( )
Trimetropim + sulfamethoxazole	( )	( )	( )	( )	( )	( )

43. Have your symptoms come back after treatment?
( ) No *end of the questionnaire*
( ) Yes *Go to the question 44*

44. Which symptoms returned after treatment? __________ *end of the questionnaire*

45. In which year did *Brucella* infection occur?
( ) List of years from 1948 or before to 2018

46. If it was not due to accidental exposure, which do you think was the cause of the brucellosis?
[ ] Ingestion of unpasteurized milk or milk products
[ ] Unprotected contact with uterine secretions from infected animals
[ ] Inhalation of aerosols from uterine secretions of infected animals
[ ] Not known

47. Which of the following symptoms did you have when you had brucellosis?

	None	1 – 3 days	3 – 7 days	7 – 15 days	15- 30 days	More than 30 days
Weakness	( )	( )	( )	( )	( )	( )
Muscle aches	( )	( )	( )	( )	( )	( )
Joint pain	( )	( )	( )	( )	( )	( )
Weight loss	( )	( )	( )	( )	( )	( )
Headaches	( )	( )	( )	( )	( )	( )
Fever	( )	( )	( )	( )	( )	( )
Diarrhea	( )	( )	( )	( )	( )	( )
Vomiting	( )	( )	( )	( )	( )	( )
Chills and sweating	( )	( )	( )	( )	( )	( )

48. Did you seek medical attention because of the symptoms?
( ) No
( ) Yes

49. Have you ever been tested for brucellosis? What was the result?

	Did not realize	Positive	Negative	Inconclusive
indirect ELISA	( )	( )	( )	( )
PCR	( )	( )	( )	( )
SAT	( )	( )	( )	( )
AAT	( )	( )	( )	( )
Coombs	( )	( )	( )	( )
2-ME	( )	( )	( )	( )
Culture	( )	( )	( )	( )

50. Did you use any antimicrobial to treat the disease?
( ) No *end of the questionnaire*
( ) Yes *Go to the question 51*

51. Which antimicrobials were used in the treatment?

	None	1 – 3 days	3 – 7 days	7 – 15 days	15- 30 days	More than 30 days
Penicillin or cephalosporin	( )	( )	( )	( )	( )	( )
Ampicillin	( )	( )	( )	( )	( )	( )
Amoxicillin + clavulanate	( )	( )	( )	( )	( )	( )
Macrolides such as azithromycin, clarithromycin, erythromycin	( )	( )	( )	( )	( )	( )
Quinolones such as ciprofloxacin, levofloxacin	( )	( )	( )	( )	( )	( )
Doxycycline	( )	( )	( )	( )	( )	( )
Other tetracyclines such as minocycline	( )	( )	( )	( )	( )	( )
Aminoglycosides such as streptomycin, gentamicin	( )	( )	( )	( )	( )	( )
Rifampicin	( )	( )	( )	( )	( )	( )
Trimetropim + sulfamethoxazole	( )	( )	( )	( )	( )	( )

52. Have your symptoms come back after treatment?
( ) No *end of the questionnaire*
( ) Yes *Go to the question 53*

53. Which symptoms returned after treatment? __________ *end of the questionnaire*

Thank you very much for your participation!

S2 Appendix: Questionário
Avaliação sobre ocorrência de brucelose e exposição acidental às vacinas B19 e RB51 entre médicos veterinários cadastrados no Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal (PNCEBT) em Minas Gerais:

Legenda: ( ) ou ○ = é possível selecionar apenas uma alternativa por pergunta
[ ] ou □ = é possível selecionar mais de uma alternativa por pergunta

1. Termo de consentimento livre esclarecido:

I - Identificação do projeto Título do trabalho experimental: Fatores de risco para brucelose como doença ocupacional em médicos veterinários em Minas Gerais. Instituição: Universidade Federal de Lavras. Pesquisadoras responsáveis: Elaine Maria Seles Dorneles e Carine Rodrigues Pereira. Telefone para contato: (35) 3829-1148 E-mails: elaine.dorneles@dmv.ufla.br ou rpcarine@gmail.com

II - Esclarecimentos: A metodologia do projeto baseia-se na aplicação do questionário “Avaliação sobre ocorrência de brucelose e exposição acidental às vacinas B19 e RB51 entre veterinários em Minas Gerais”, via Google forms, encaminhado aos participantes por e-mail e telefone, fornecidos pelo IMA juntamente com o nome e a cidade de cada profissional. Não há riscos ou desconfortos previsíveis para a sua participação na pesquisa, a não ser o tempo dispendido de aproximadamente 20 minutos necessários para responder o questionário. Todas as informações coletadas serão utilizadas apenas para análises estatísticas e os dados pessoais serão mantidos estritamente confidenciais. Você não terá nenhuma despesa ao participar do estudo e poderá deixar de participar ou retirar seu consentimento a qualquer momento, sem precisar justificar, e não sofrerá qualquer prejuízo. Não há nenhum valor econômico, a receber ou a pagar, pela sua participação. É garantido seu direito de esclarecimentos adicionais sobre o estudo e suas consequências, enfim, tudo o que você queira saber antes, durante e depois da sua participação.

III - Participação voluntária: A sua participação em qualquer tipo de pesquisa é voluntária. Em caso de dúvida quanto aos seus direitos, escreva ou ligue para o Comitê de Ética em Pesquisa em Seres Humanos da UFLA. Endereço – Campus Universitário da UFLA, Pró-reitoria de pesquisa, COEP, caixa postal 3037, telefone: (35) 3829-5182. Declaro que li e entendi todos os procedimentos que serão realizados neste trabalho. Declaro também, que fui informado que posso desistir a qualquer momento. Assim, aceito participar como voluntário do projeto de pesquisa descrito acima. Uma cópia deste termo será enviado para seu e-mail automaticamente.

( ) Aceito
( ) Não aceito *como você não concorda com os termos, não poderá continuar participando da pesquisa: fim do questionário*

2. Email: ____________

3. Em qual cidade você mora?
( ) Lista dos 853 municípios mineiros

4. Ano de nascimento:
( ) Lista de anos de 1948 a 1996

5. Gênero:
( ) Masculino
( ) Feminino

6. Há quanto tempo trabalha na profissão (anos)?
( ) Menos de um ano
( ) Lista de 1 a 47 anos
( ) 48 anos ou mais

7. Ano em que se cadastrou como vacinador no PNCEBT:
( ) Lista de anos de 2002 a 2018

8. Em quais das regiões produtoras de bovinos você atua profissionalmente?
[ ] Alto Paranaíba
[ ] Central
[ ] Leste
[ ] Noroeste, Norte e Nordeste
[ ] Sul e Sudeste
[ ] Triângulo Mineiro
[ ] Zona da Mata

9. Além de cadastrado, também é habilitado para realização de diagnóstico de brucelose e tuberculose?
( ) Não
( ) Sim

10. Trabalha principalmente com:
( ) Bovinos/Bubalinos de leite
( ) Bovinos/Bubalinos de corte
( ) Pequenos ruminantes
( ) Cães e gatos
( ) Equinos
( ) Serviços administrativos
( ) Inspeção
( ) Defesa sanitária animal
( ) Outros: ___

11. Qual seu vínculo empregatício?
( ) Autônomo
( ) Empresa privada
( ) Servidor público

12. Marque a quantidade de procedimentos realizados em bovinos/ bubalinos nos últimos seis meses:

	0	1 - 5	6 - 10	≥ 11
Partos	( )	( )	( )	( )
Remoção manual da placenta retida	( )	( )	( )	( )
Vacinação contra brucelose	( )	( )	( )	( )
Parto premature ou atendimento de abortos	( )	( )	( )	( )

13. Qual tipo de equipamento de proteção individual (EPI) utilizou durante estes procedimentos?

	Sempre	Às vezes	Nunca
Luvas	( )	( )	( )
Casaco	( )	( )	( )
Óculos de proteção	( )	( )	( )
Máscara	( )	( )	( )

14. Caso alguma resposta seja às vezes ou nunca, qual o motivo da não utilização do equipamento?

	Não se aplica	Dificulta a realização do procedimento	Falta de tempo	Falta de hábito	Não possuo o equipamento	Não acho importante
Luvas	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Casaco	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Óculos de proteção	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Máscara	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

15. Como é realizado o descarte dos materiais utilizados na vacinação contra brucelose?
 [ ] Lixo comum da propriedade rural
 [ ] Lixo comum urbano
 [ ] Lixo infectante
 [ ] Retorna os materiais utilizados para a loja agropecuária
 [ ] Enterra na propriedade

16. Na sua opinião, quais as principais formas de transmissão da brucelose?
 [ ] Contato desprotegido com produtos de abortos contaminados
 [ ] Contato direto com saliva de bovinos/bubalinos doentes
 [ ] Ingestão de leite e derivados não submetidos à tratamento térmico
 [ ] Auto inoculação com as vacinas B19 e RB51
 [ ] Ingestão de carnes mal cozidas

17. Na sua opinião, a brucelose é uma doença que provoca no homem:
 [ ] Sinais clínicos principalmente reprodutivos, assim como nos animais
 [ ] Dores nas articulações, sudorese, febre e calafrios
 [ ] Andar cambaleante e desorientação mental nos primeiros dias da infecção
 [ ] Endocardites e orquites podem ocorrer em casos graves

18. Você consumiu leite ou derivados sem tratamento térmico nos últimos 6 meses?
 ( ) Não
 ( ) Sim

19. Você possui vacinadores cadastrados sob sua responsabilidade?
 ( ) Não *vá para a pergunta 27*
 ( ) Sim *vá para a pergunta 20*

20. O vacinador recebeu algum tipo de treinamento para realizar a vacinação de bovinos/bubalinos com B19 e RB51?
 ( ) Não
 ( ) Sim

21. Quais destes equipamentos o vacinador utiliza durante a manipulação das vacinas B19 e RB51?

	Sim	Não	Não sei
Luvas	( )	( )	( )
Casaco	( )	( )	( )
Óculos de proteção	( )	( )	( )
Máscara	( )	( )	( )

22. O vacinador já relatou algum tipo de exposição acidental às vacinas B19 ou RB51?
 ( ) Não *vá para a pergunta 27*
 ( ) Sim *vá para a pergunta 23*
 ( ) Não sei *vá para a pergunta 27*

23. A qual vacina o vacinador foi exposto? Quantas vezes?

	Nenhuma vez	1 vez	2 vezes	Mais de 2 vezes
B19	( )	( )	( )	( )
RB51	( )	( )	( )	( )

24. O vacinador procurou atendimento médico após a exposição acidental às vacinas B19 ou RB51?
 ( ) Não
 ( ) Sim
 ( ) Não sei

25. O vacinador apresentou algum sintoma após a exposição acidental às vacinas B19 ou RB51?
( ) Não *vá para a pergunta 27*
( ) Sim *vá para a pergunta 26*
( ) Não sei *vá para a pergunta 27*

26. Quais dos seguintes sintomas o vacinador apresentou?

	Sim	Não	Não sei
Dor no local da inoculação	( )	( )	( )
Fraqueza	( )	( )	( )
Dores musculares	( )	( )	( )
Dores articulares	( )	( )	( )
Perda de peso	( )	( )	( )
Dores de cabeça	( )	( )	( )
Febre	( )	( )	( )
Diarreia	( )	( )	( )
Vômito	( )	( )	( )
Calafrios e sudorese	( )	( )	( )

27. Você já teve brucelose?
( ) Não *vá para a pergunta 29*
( ) Sim *vá para a pergunta 28*

28. A brucelose foi causada por exposição acidental às vacinas B19 ou RB51?
( ) Não *vá para a pergunta 45*
( ) Sim *vá para a pergunta 30*

29. Você já foi exposto acidentalmente à vacina antibrucélica?
( ) Não *fim do questionário*
( ) Sim *vá para a pergunta 28*

30. Em que ano ocorreu sua primeira exposição acidental às vacinas B19 ou RB51?
( ) 1994 ou antes
( ) Lista de anos de 1995 a 2018

31. Qual tipo de exposição ocorreu?
[ ] Penetração da agulha
[ ] Aerossóis da vacina na mucosa ocular
[ ] Aerossóis da vacina com mucosa oronasal
[ ] Contato da vacina com pele íntegra
[ ] Contato da vacina com pele lesionada
[ ] Outro ____

32. A qual vacina você foi exposto? Quantas vezes?

	Nenhuma vez	1 vez	2 vezes	Mais de 2 vezes
B19	( )	( )	( )	( )
RB51	( )	( )	( )	( )

33. Em qual momento ocorreu o acidente?
[ ] Manipulação do frasco da vacina
[ ] Vacinação do gado
[ ] Desmontagem da seringa
[ ] Descarte dos materiais
[ ] Ao reencapar a agulha
[ ] Outro _____

34. Quais equipamentos de proteção individual você usava quando foi exposto à(s) vacina(s) antibrucélica(s)?
[ ] Luvas
[ ] Máscara
[ ] Casaco / Macacão de manga longa
[ ] Óculos de proteção
[ ] Nenhuma proteção
[ ] Outro ___

35. Qual você acha que foi o motivo da exposição acidental à vacina antibrucélica?
[ ] Desconhecimento sobre os riscos da vacina
[ ] Falta de infra estrutura da propriedade para realizar a vacinação
[ ] Temperamento do animal
[ ] Medidas de proteção inadequadas
[ ] Outro ___

36. O que você fez após a exposição?
[ ] Lavou o local
[ ] Desinfecção do local com antisséptico
[ ] Procurou atendimento médico
[ ] Auto medicação
[ ] Nada
[ ] Outro ___

37. Em caso de automedicação após a exposição à vacina antibrucélica, qual foi o fármaco e protocolo utilizados? Caso não tenha realizado a automedicação, escreva NA. ___________

38. Quais sintomas você apresentou quando foi exposto à vacina antibrucélica?

	Nenhum dia	1 – 3 dias	3 – 7 dias	7 – 15 dias	15- 30 dias	Mais de 30 dias
Dor no local da inoculação	( )	( )	( )	( )	( )	( )
Fraqueza	( )	( )	( )	( )	( )	( )
Dores musculares	( )	( )	( )	( )	( )	( )
Dores articulares	( )	( )	( )	( )	( )	( )
Perda de peso	( )	( )	( )	( )	( )	( )
Dores de cabeça	( )	( )	( )	( )	( )	( )
Febre	( )	( )	( )	( )	( )	( )
Diarreia	( )	( )	( )	( )	( )	( )
Vômito	( )	( )	( )	( )	( )	( )
Calafrios e sudorese	( )	( )	( )	( )	( )	( )

39. Você procurou atendimento médico devido aos sintomas?
( ) Não
( ) Sim

40. Você fez algum teste para diagnosticar a possibilidade de ter se infectado com brucelose devido à exposição à vacina antibrucélica? Qual foi o resultado?

	Não realizei	Positivo	Negativo	Inconclusivo
ELISA indireto	( )	( )	( )	( )
PCR	( )	( )	( )	( )
SAT	( )	( )	( )	( )
AAT	( )	( )	( )	( )
Coombs	( )	( )	( )	( )
2-ME	( )	( )	( )	( )
Cultura	( )	( )	( )	( )

41. Você utilizou antibiótico para tratamento pós exposição à vacina antibrucélica?
( ) Não *fim do questionário*
( ) Sim *vá para a pergunta 42*

42. Quais antibióticos foram utilizados no tratamento?

	Nenhum dia	1 – 3 dias	3 – 7 dias	7 – 15 dias	15- 30 dias	Mais de 30 dias
Penicilina ou cefalosporina	( )	( )	( )	( )	( )	( )
Ampicilina	( )	( )	( )	( )	( )	( )
Amoxicilina + clavulanato	( )	( )	( )	( )	( )	( )
Macrolídeos como azitromicina, claritromicina, eritromicina	( )	( )	( )	( )	( )	( )
Quinolonas como ciprofloxacina, levofloxacina	( )	( )	( )	( )	( )	( )
Doxiciclina	( )	( )	( )	( )	( )	( )
Outras tetraciclinas como minociclina	( )	( )	( )	( )	( )	( )
Aminoglicosídeos como estreptomicina, gentamicina	( )	( )	( )	( )	( )	( )
Rifampicina	( )	( )	( )	( )	( )	( )
Trimetropim + sulfametoxazol	( )	( )	( )	( )	( )	( )

43. Seus sintomas voltaram a aparecer após o tratamento?
( ) Não *fim do questionário*
( ) Sim *vá para a pergunta 44*

44. Quais sintomas voltaram a aparecer após o tratamento? __________ *fim do questionário*

45. Em que ano ocorreu sua infecção por brucelose?
( ) Lista de anos de 1948 ou antes a 2018

46. Se não foi devido à exposição acidental, qual você acha que foi a causa da infecção por brucelose?
[ ] Consumo de leite e derivados não submetidos a tratamento térmico
[ ] Contato desprotegido com secreções uterinas de animais infectados
[ ] Inalação de aerossóis de secreções uterinas de animais infectados
[ ] Não sei

47. Quais dos seguintes sintomas você apresentou quando teve brucelose?

	Nenhum dia	1 – 3 dias	3 – 7 dias	7 – 15 dias	15- 30 dias	Mais de 30 dias
Fraqueza	( )	( )	( )	( )	( )	( )
Dores musculares	( )	( )	( )	( )	( )	( )
Dores articulares	( )	( )	( )	( )	( )	( )
Perda de peso	( )	( )	( )	( )	( )	( )
Dores de cabeça	( )	( )	( )	( )	( )	( )
Febre	( )	( )	( )	( )	( )	( )
Diarreia	( )	( )	( )	( )	( )	( )
Vômito	( )	( )	( )	( )	( )	( )
Calafrios e sudorese	( )	( )	( )	( )	( )	( )

48. Você procurou atendimento médico devido aos sintomas?
( ) Não
( ) Sim

49. Você fez algum teste para diagnosticar a doença? Qual foi o resultado?

	Não realizei	Positivo	Negativo	Inconclusivo
ELISA indireto	( )	( )	( )	( )
PCR	( )	( )	( )	( )
SAT	( )	( )	( )	( )
AAT	( )	( )	( )	( )
Coombs	( )	( )	( )	( )
2-ME	( )	( )	( )	( )
Cultura	( )	( )	( )	( )

50. Você utilizou algum antibiótico para tratamento da doença?
( ) Não *fim do questionário*
( ) Sim *vá para a pergunta 51*

51. Quais antibióticos foram utilizados no tratamento?

	Nenhum dia	1 – 3 dias	3 – 7 dias	7 – 15 dias	15- 30 dias	Mais de 30 dias
Penicilina ou cefalosporina	( )	( )	( )	( )	( )	( )
Ampicilina	( )	( )	( )	( )	( )	( )
Amoxicilina + clavulanato	( )	( )	( )	( )	( )	( )
Macrolídeos como azitromicina, claritromicina, eritromicina	( )	( )	( )	( )	( )	( )
Quinolonas como ciprofloxacina, levofloxacina	( )	( )	( )	( )	( )	( )
Doxiciclina	( )	( )	( )	( )	( )	( )
Outras tetraciclinas como minociclina	( )	( )	( )	( )	( )	( )
Aminoglicosídeos como estreptomicina, gentamicina	( )	( )	( )	( )	( )	( )
Rifampicina	( )	( )	( )	( )	( )	( )
Trimetropim + sulfametoxazol	( )	( )	( )	( )	( )	( )

52. Seus sintomas voltaram a aparecer após o tratamento?
( ) Não *fim do questionário*
( ) Sim *vá para a pergunta 53*

53. Quais sintomas voltaram a aparecer após o tratamento? __________ *fim do questionário*

Muito obrigada pela sua participação!

S3 Appendix: Variables analysed

Variable	Description	Logistic regression analysis	Descriptive analysis
X1*	City of residence – later inserted in its corresponding strata of bovine producing region	IV	Alto Paranaíba – 36 (10.94%) Central – 98 (29.79%) Leste – 18 (5.47%) Noroeste, Norte and Nordeste – 33 (10.03%) Sul and Sudoeste – 72 (21.88%) Triângulo Mineiro – 35 (10.64%) Zona da Mata – 37 (11.25%)
X2*	Year of birth – later transformed into age (years)	IV	Median – 37.00 Average – 40.63 Interquartile range – 18.00 Standard deviation – 12.13
X3*	Gender	IV	Female – 56 (17.02%) Male – 273 (82.98%)
X4*	Professional experience (years)	IV	Median – 10.00 Average – 14.49 Interquartile range – 14.00 Standard deviation – 11.65
X5*	PNCEBT registration (year)	IV	Median – 2010 Average – 2009.80 Interquartile range – 10.00 Standard deviation – 5.37
X6*	It is enabled in PNCEBT to perform brucellosis diagnosis	IV	Yes – 144 (43.77%) No – 185 (56.23%)
X7*	Main field of work	IV	Dairy cattle – 214 (65.05%) Beef cattle – 59 (17.93%) Others – 56 (17.02%)
X8*	Employment relationship	IV	Self-employed – 235 (71.43%) Private company – 67 (20.36%) Public server – 27 (8.21%)
X9*	Deliveries performed in the last six months (none, 1 - 5, 6 - 10 or ≥ 11) – later turned into yes or no	IV	Yes – 240 (72.95%) No – 89 (27.05%)
X10*	Manual placenta removal performed in the last six months (none, 1 - 5, 6 - 10 or ≥ 11) – later turned into yes or no	IV	Yes – 179 (54.41%) No – 150 (45.59%)
X11*	S19 or RB51 vaccination carried out in the last six months (none, 1 - 5, 6 - 10 or ≥ 11) – later turned into yes or no	IV	Yes – 309 (93.92%) No – 20 (6.08%)
X12*	Abortions care performed in the last six months (none, 1 - 5, 6 - 10 or ≥ 11) – later turned into yes or no	IV	Yes – 177 (53.80%) No – 152 (46.20%)
X13*	Frequency of gloves use (always, sometimes, never) – later transformed into personal protective equipment (PPE) use	IV	Always – 277 (84.19%) Sometimes – 41 (12.46%) Never – 11 (3.34%)
X14*	Frequency of coat use (always, sometimes, never) – later transformed into personal PPE use	IV	Always – 133 (40.43%) Sometimes – 80 (24.32%) Never – 116 (35.26%)
X15*	Frequency of goggles use (always, sometimes, never) – later transformed into personal PPE use	IV	Always – 70 (21.28%) Sometimes – 52 (15.81%)

			Never – 207 (62.92%)
X16*	Frequency of mask use (always, sometimes, never) – later transformed into personal PPE use	IV	Always – 30 (9.12%) Sometimes – 55 (16.72%) Never – 244 (74.16%)
X17*	Reason for not using gloves (list of possible causes of low adherence to the use of the equipment) [possibility to select more than one option]	-	Makes it difficult to carry out the procedure – 47 (14.11%) Lack of habit – 16 (4.80%) Lack of time – 3 (0.90%) I do not consider it important – 4 (1.20%) I do not own the equipment – 2 (0.60%) Not applicable – 261 (78.38%)
X18*	Reason for not using coat (list of possible causes of low adherence to the use of the equipment) [possibility to select more than one option]	-	Makes it difficult to carry out the procedure – 61 (17.89%) Lack of habit – 69 (20.23%) Lack of time – 9 (2.64%) I do not consider it important – 20 (5.87%) I do not own the equipment – 37 (10.85%) Not applicable – 145 (42.52%)
X19*	Reason for not using goggles (list of possible causes of low adherence to the use of the equipment) [possibility to select more than one option]	-	Makes it difficult to carry out the procedure – 57 (16.81%) Lack of habit – 129 (38.05%) Lack of time – 3 (0.88%) I do not consider it important – 21 (6.19%) I do not own the equipment – 33 (9.73%) Not applicable – 96 (28.32%)
X20*	Reason for not using mask (list of possible causes of low adherence to the use of the equipment) [possibility to select more than one option]	-	Makes it difficult to carry out the procedure – 55 (16.18%) Lack of habit – 155 (45.59%) Lack of time – 3 (0.88%) I do not consider it important – 36 (10.59%) I do not own the equipment – 30 (8.82%) Not applicable – 61 (17.94%)
X21*	Form of anti-*Brucella* vaccine disposal (infection waste, general waste, bury in the property…)	-	General waste from rural property – 93 (28.27%) Urban general waste – 38 (11.55%) Infectious waste – 104 (31.61%) Return to the agricultural store – 28 (8.51%) Bury in the rural property – 37 (11.25%) Burn – 29 (8.81%)
X22*	Knowledge about brucellosis transmission – later transformed into good, average or poor	IV	Good – 156 (47.42%) Average – 171 (51.98%) Poor – 2 (0.61%)
X23*	Knowledge about brucellosis symptoms – later transformed into good, average or poor	IV	Good – 275 (83.59%) Average – 37 (11.25%) Poor – 17 (5.17%)
X24*	Consumption of raw milk or other dairy products in the last six months	IV	Yes – 105 (31.91%) No – 224 (68.09%)
X25*	There is some vaccinator registered under its responsibility	IV	Yes – 85 (25.84%) No – 244 (74.16%)
X26	Vaccinator received training handle S19 or RB51 vaccines	-	Yes – 81 (95.29%) No – 4 (4.71%)
X27	Frequency of gloves use by the vaccinator	-	Yes – 81 (95.29%) No – 2 (2.35%) Not known – 2 (2.35%)

X28	Frequency of coat use by the vaccinator	-	Yes – 26 (30.59%) No – 45 (52.94%) Not known – 14 (16.47%)
X29	Frequency of goggles use by the vaccinator	-	Yes – 25 (29.41%) No – 44 (51.76%) Not known – 16 (18.82%)
X30	Frequency of mask use by the vaccinator	-	Yes – 15 (17.65%) No – 55 (64.71%) Not known – 15 (17.65%)
X31	The vaccinator have ever been exposed to anti-*Brucella* vaccine	-	Yes – 11 (12.94%) No – 70 (82.35%) Not known – 4 (12.94%)
X32	Anti-*Brucella* vaccine to which the vaccinator was exposed (how many times + S19 or RB51)	-	S19: Once – 7 (63.64%) Twice – 1 (9.09%) More than twice – 3 (27.27%) RB51: None – 11 (100.00%)
X33	Did the vaccinator seek medical attention after the accidental exposition	-	Yes – 6 (54.55%) No – 3 (27.27%) Not known – 2 (18.18%)
X34	Vaccinator's clinical signs after S19 or RB51 exposure (list of symptoms + yes, no or not known)	-	Yes: Pain at the site of inoculation – 2 (100.00%) Weakness – 1 (50.00%) Muscle pain – 1 (50.00%) Joint pain – 0 (0.00%) Weight loss – 0 (0.00%) Headaches – 1 (50.00%) Fever – 2 (100.00%) Diarrhea – 0 (0.00%) Vomiting – 0 (0.00%) Chills and sweating – 0 (0.00%) No – 8 (72.73%) Not known – 1 (9.09%)
X35*	The veterinary has ever had brucellosis (yes or no)		Yes – 15 (4.56%) No – 314 (95.44%)
X36*	Was brucellosis caused by accidental exposure to S19 or RB51 (yes or no)		Yes – 7 (46.67%) No – 8 (53.33%)
X37*	Veterinary has ever been accidentally exposed to anti-*Brucella* vaccine (yes or no)	DV	Yes – 108 (32.83%) No – 221 (67.17%)
X38	When the first accidental S19 or RB51 exposition occur (year)	-	Median – 2013 Average – 2010 Interquartile range – 10.00 Standard deviation – 7.85
X39	Type of exposure (needlestick injury, vaccine splashing into mucosa, skin contact…) [possibility to select more than one option]	-	Penetration of needle – 60 (37.74%) Splashing of vaccine content into eyes – 33 (20.75%) Splashing of vaccine content into oral/nasal mucosa – 10 (6.29%) Contact of vaccine content to not wounded skin – 49 (30.82%) Contact of vaccine content to wounded skin – 7 (4.40%)

X40	Anti-*Brucella* vaccine to which the veterinary was exposed (how many times + S19 or RB51)	-	S19: Once – 49 (45.37%) Twice – 30 (27.78%) More than twice – 25 (23.15%) RB51: Once – 1 (0.93%) More than twice – 1 (0.93%) S19 and RB51: Once to S19 and once to RB51 – 1 (0.93%) More than twice to S19 and once to RB51 – 1 (0.93%)
X41	At what point did the accident occur (livestock vaccination, re-encaping the needle…) [possibility to select more than one option]	-	Vaccine bottle manipulation – 61 (45.52%) Livestock vaccination – 54 (40.30%) Disassembling the syringe – 7 (5.22%) Disposal of materials – 5 (3.73%) When re-capping the needle – 7 (5.22%)
X42	Which personal protective equipment was being used during accidental exposition (gloves, coat…) [possibility to select more than one option]	-	None – 14 (12.96%) One PPE – 52 (48.15%) Two PPE – 36 (33.33%) Three PPE – 5 (4.63%) All PPE – 1 (0.93%)
X43	Probable reason for accidental S19 or RB51 exposition (animal's temperament, lack of infrastructure…)	-	Lack of knowledge about vaccine risks – 2 (1.61%) Lack of infrastructure to carry out the vaccination – 43 (34.68%) Animal's temperament – 22 (17.74%) Inadequate protection measures – 33 (26.61%) Lack of attention or hurry – 9 (7.26%) Pressure in the vaccine bottle when needle is inserted – 6 (4.84%) Other – 9 (7.26%)
X44	Measure taken immediately after the accidental exposure (washed the place, self-medication, nothing…)	-	Washed the place – 81 (47.09%) Disinfection of the site with antiseptic – 56 (32.56%) Seek medical attention – 19 (11.05%) Self-medication – 7 (4.07%) Amoxicillin – 1 (14.29%) Doxycycline – 2 (28.57%) Rifampicin + Tetracycline – 1 (14.29%) Sulfonamide – 1 (14.29%) Local tetracycline (veterinary product) – 2 (28.57%) Nothing – 6 (3.49%) Cauterization – 3 (1.74%)
X45	Veterinary's clinical signs after anti-*Brucella* vaccine exposure (list of symptoms)	-	Pain at the site of inoculation: Yes – 36 (33.33%) No – 72 (66.67%) Weakness: Yes – 2 (1.85%) No – 106 (98.15%) Muscle pain: Yes – 4 (3.70%) No –104 (96.30%) Joint pain: Yes – 4 (3.70%) No –104 (96.30%)

			Weight loss: Yes – 0 (0.00%) No – 108 (100.00%) Headaches: Yes – 2 (1.85%) No – 106 (98.15%) Fever: Yes – 2 (1.85%) No – 106 (98.15%) Diarrhea: Yes – 0 (0.00%) No – 108 (100.00%) Vomiting: Yes – 0 (0.00%) No – 108 (100.00%) Chills and sweating: Yes – 2 (1.85%) No – 106 (98.15%)
X46	Did the veterinary seek medical attention after the accidental exposition	-	Yes – 17 (15.74%) No – 91 (84.26%)
X47	Was any diagnostic method used to check for post-exposure infection (list of methods + possible results)	-	Indirect ELISA: Inconclusive – 1 (0.93%) Did not realize – 86 (79.63%) Negative – 16 (14.81%) Positive – 5 (4.63%) PCR: Did not realize – 99 (91.67%) Negative – 9 (8.33%) SAT: Did not realize – 102 (94.44%) Negative – 6 (5.56%) AAT: Did not realize – 87 (80.56%) Negative – 19 (17.59%) Positive – 2 (1.85%) Coombs: Did not realize – 104 (96.30%) Negative – 4 (3.70%) 2-ME: Did not realize – 102 (94.44%) Negative – 6 (5.56%) Culture: Did not realize – 100 (92.59%) Negative – 7 (6.48%) Positive – 1 (0.93%)
X48	Was any antimicrobial used after anti-*Brucella* vaccine exposure (list of drugs + therapeutic protocol)	-	Yes – 20 (18.52%) Penicillin or cephalosporin – 1 (5.00%) Penicillin or cephalosporin and Amoxicillin + clavulanate – 1 (5.00%) Amoxicillin + clavulanate and other tetracyclines – 1 (5.00%) Macrolides – 1 (5.00%)

			Quinolones – 1 (5.00%) Doxycycline and Aminoglycosides and Rifampicin – 1 (5.00%) Doxycycline and Rifampicin and Trimetropim + sulfamethoxazole – 1 (5.00%) Doxycycline and Rifampicin – 2 (10.00%) Doxycycline – 6 (30.00%) Other tetracyclines – 2 (10.00%) Aminoglycosides – 1 (5.00%) Rifampicin and Trimetropim + sulfamethoxazole – 1 (5.00%) Trimetropim + sulfamethoxazole – 1 (5.00%) No: 88 (81.48%)
X49	There was a relapse of any symptoms arising from anti-*Brucella* vaccine exposure	-	Yes – 2 (10.00%) Joint pain – 1 (10.00%) Allergy – 1 (10.00%) No – 18 (90.00%)
X50	When *Brucella abortus* infection occurred (year)	-	Median – 2010 Average – 2006.40 Interquartile range – 11.50 Standard deviation – 10.22
X51	Probable cause of its *B. abortus* infection (ingestion of raw milk, unprotect contact with sick animals…)	-	Accidental exposure to anti-Brucella vaccine strains – 7 (46.67%) Unprotected contact with uterine secretions from infected animals – 6 (40.00%) Ingestion of raw milk and unprotected contact with uterine secretions from infected animals during cirurgical procedure – 1 (6.67%) Not known – 1 (6.67%)
X52	Veterinary's clinical signs during *B. abortus* infection (list of symptoms + duration in days)	-	Weakness: Yes – 5 (33.33%) No – 10 (66.67%) Muscle pain: Yes – 7 (46.67%) No – 8 (53.33%) Joint pain: Yes – 7 (46.67%) No – 8 (53.33%) Weight loss: Yes – 2 (13.33%) No – 13 (86.67%) Headaches: Yes – 3 (20.00%) No – 12 (80.00%) Fever: Yes – 4 (26.67%) No – 11 (73.33%) Diarrhea: Yes – 1 (6.67%) No – 14 (93.33%) Vomiting: Yes – 0 (0.00%) No – 15 (100.00%) Chills and sweating: Yes – 5 (33.33%) No – 10 (66.67%)

X53	Did the veterinary seek medical attention due brucellosis	-	Yes – 8 (53.33%) No – 7 (46.67%)
X54	Was any diagnostic method used to check *B. abortus* infection (list of methods + possible results)	-	Indirect ELISA: Did not realize – 6 (40.00%) Positive – 9 (60.00%) PCR: Did not realize – 14 (93.33%) Positive – 1 (6.67%) SAT: Did not realize – 15 (100.00%) AAT: Did not realize – 9 (60.00%) Negative – 1 (6.67%) Positive – 5 (33.33%) Coombs: Did not realize – 15 (100.00%) 2-ME: Did not realize – 15 (100.00%) Culture: Did not realize – 13 (86.67%) Negative – 1 (6.67%) Positive – 1 (6.67%)
X55	Was any antimicrobial used in the treatment of brucellosis (list of drugs + therapeutic protocol)	-	Yes – 9 (60.00%) Penicillin or cephalosporin and Aminoglycoside – 1 (6.67%) Doxycycline, Rifampicin and Aminoglycosides – 1 (6.67%) Doxycycline, Rifampicin and Trimethoprim + sulfamethoxazole – 1 (6.67%) Doxycycline and Rifampicin – 3 (20.00%) Doxycycline – 1 (6.67%) Other tetracycline – 1 (6.67%) Aminoglycoside – 1 (6.67%) No: 6 (40.00%)
X56	There was a relapse of any symptoms arising from *B. abortus* infection (yes or no)	-	Yes – 2 (13.33%) Joint pain – 1 (6.67%) Allergy – 1 (6.67%) No – 13 (86.67%)

* = Required questions
IV = Independent variable of accidental exposure to anti-*Brucella* vaccines model
DV = Dependent variable of accidental exposure to anti-*Brucella* vaccines model
PPE = Personal protective equipment
PNCEBT = National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis

2. FINAL CONSIDERATIONS

Information are usually raised out within universities and passed on to public agencies responsible for health promotion, whether human, animal or environmental. Then, these agencies develop strategic actions that will be put into practice by a multidisciplinary staff. The work developed by this study can positively impact an entire community, since veterinarians have a very important role in this scenario. However, in order to work into health promotion and knowledge dissemination, this occupational group obviously must first be healthy and aware of all aspects related to the dynamics of the most varied zoonoses, among them brucellosis.

The high prevalence of accidental exposure to S19 and RB51 vaccine strains identified in veterinarians from Minas Gerais state and the strong occupational character of brucellosis in these professionals all around the world are the main findings of this dissertation. Prevention of *Brucella* spp. infection among humans can be achieved by means of continuing education measures after university degree and training for the proper use of personal protective equipment. Thereby, veterinarians will be able to experience and effectively act in the One Health promotion.