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Chapter 4

Seroprevalence of brucellosis and assessment of zoonotic risk factors posed to abattoir workers in Gauteng abattoirs: a One Health Approach.

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4.1 Abstract

This study was conducted to obtain evidence-based data for the prospect of conducting a seroprevalence study of brucellosis among abattoir workers and managers in the Gauteng province of South Africa. Fourteen abattoirs were visited in the first phase of sampling, during which 14 abattoir managers/owners and 143 abattoir workers who consented, were interviewed to assess risk factors that could predispose exposure to occupational zoonotic infections. In the second phase, 103 consenting abattoir workers were interviewed using a structured questionnaire to assess the knowledge acquired during the zoonoses sensitization campaign conducted in the first phase. Also, in the second phase, the occurrence of brucellosis among the 103 abattoir workers, managers and meat inspectors was determined using serological assays (Rose Bengal test (RBT), BrucellaCap and IgG-ELISA) at six abattoirs where brucellosis-positive slaughter cattle were identified of which three were multispecies abattoirs where sheep was also slaughtered.

Of the 14 managers interviewed in the first phase, 71.4% (10/14) claimed had knowledge of brucellosis, cysticercosis, leptospirosis, Q-fever, tuberculosis and toxoplasmosis, while, 28.6% (4/14) had knowledge of five of the diseases in question except Q-fever. All the 14 managers indicated to have educated their workers on the danger of zoonoses, provided them with personal protective equipment (PPE) and enforced PPE usage during work. The managers confirmed that none of the abattoir workers was diagnosed with any of the zoonoses in the questionnaire prior to the interview. Of the 14 managers, 28.6% (4/14) confirmed that

some workers engaged in risky practices sometimes while at work. For the workers' activities at the abattoirs, 6.30% (9/143) indicated that they ate raw meat; 1.40% (2/143) had stolen and consumed condemned meat; 84.0% (120/143) had hand cut injuries at least once; 91.0% (130/143) had experienced splash of blood or fluid on their faces; 90.2% (129/143) did not use facial protective goggles, 69.9% (100/143) did not use facial mask; 58.0% (83/143) did not wear hand gloves while working; 38.5% (55/143) ate breakfast while wearing PPE; 67.1% (96/143) had experienced symptoms of febrile illness (fever and flu); 88.1% did not seek medical help whenever they felt symptoms of illness, and 37.1% (53/143) believed and had perception of not acquiring animal diseases while working at the abattoir. All abattoir managers and workers were sensitized on zoonoses in question, the causes, clinical signs and symptoms in animals and humans, and the preventive measures for their protection at the facilities. In the second interview and sero-testing phase to test the retention of knowledge on zoonoses in question, a knowledge gap among the respondents was detected, as only 26.2%, 8.8%, 15.5%, 11.7%, 88.3% and 3.9% of respondents could remember knowledge of brucellosis, cysticercosis, leptospirosis, Q-fever, tuberculosis and toxoplasmosis, respectively. New methods of knowledge acquisition should be explored to breach the knowledge gap among abattoir workers. In this second phase, of the 103 respondents tested, the overall distribution and estimated seroprevalence in series for exposure by *Brucella* spp. was 21 (20.4%, 95%CI=13.1-29.5) using RBT, BrucellaCap and IgG-ELISA; with RBT was 13 (12.6%, 95%CI=6.89-20.6), BrucellaCap was 9 (8.74%, 95%CI=4.07-15.9) and IgG-ELISA was 18 (17.5%, 95%CI=10.7-26.2). A multiple logistic regression model identified the risk factors in the study such as sex, job description, number of days at work and working on farms away from the abattoirs to be insignificantly ($P>0.05$) associated with human brucellosis among the abattoir workers in Gauteng province. In conclusion, our study has determined for the first time in South Africa, the occurrence of antibodies against brucellosis among abattoir workers, which could be because of infection or previous exposure to *Brucella*. This underscores the fact that the abattoir facilities can be used for active and passive surveillance of diseases of public health and economic importance. The evidence-based data provided by this study will be invaluable to policy makers. We recommend the implementation of brucellosis testing of abattoir workers country wide and availability of test data from abattoir managers to establish base-line data that could indicate previous exposure, and that can be used to mitigate and appropriate preventive practices to reduce the infection rate among these workers as well as provide insight into the magnitude of infections by *Brucella* spp. among the abattoir workers.

4.2 Introduction

Over the years, there have been attempts to estimate the global health burden of various populations at the local or international levels (Driscoll et al., 2005). About 25% of human deaths globally are attributable to infectious diseases (WHO, 2000), and these account for 29 out of the 96 major causes of human morbidity and mortality, as indicated by the World Health Organization and the World Bank (Murray and Lopez, 1996). Many emerging and re-emerging diseases are zoonotic in nature, as such, they are naturally transmissible between animals and humans (Morse, 2001, Oaks Jr et al., 1992). These emerging diseases tend to have appeared for the first time in the human population, but are now expanding into areas where they were never found before and the incidence is increasing (Taylor et al., 2001). It has been established that of all the 1,415 known human infectious pathogens, 61% of them are zoonotic, and 75% of the emerging or re-emerging diseases are also thought to be zoonotic (Taylor et al., 2001). Of these emerging zoonotic pathogens, 35% and 61% are known to be transmitted through direct and indirect contact, respectively. Of these zoonoses, 22% are known to be transmitted through vectors, while 6% account for the unknown route of transmission (Taylor et al., 2001). These estimates have potential advantages for policy makers to proffer solutions in areas that need urgent attention to mitigate issues of morbidity and mortality of workers, with regards to occupational hazards like zoonotic infections at abattoir facilities and animal farms (Battelli, 2008, Driscoll et al., 2005).

Brucellosis is one of the neglected zoonotic diseases and it is endemic in most of the developing countries (Franc et al., 2018). It is an important zoonosis that infects humans, and about half a million people are thought to be infected annually around the world (Corbel, 1997, Godfroid et al., 2005, Lopes et al., 2010). This type of zoonosis causes a huge disease burden on infected individuals in terms of high socioeconomic and financial impact (Franc et al., 2018, McDermott et al., 2013). This impact is compounded by the fact that clinical signs and symptoms of human brucellosis vary from months to years and the prolonged episodes of clinical signs cause additional losses of time and money owing to disability (Abdou, 2000, Battelli, 2008, Zajtchuk and Bellamy, 1997). The risk of brucellosis infection is relatively high among individuals who work in abattoir facilities, meat packaging industries, farm workers and the occupational hazard posed to these susceptible workers is highly significant (Young, 1995). Apart from abattoirs being a facility where apparently healthy animals are slaughtered for the production of wholesome meat for human consumption, the facilities can also be used for monitoring effectiveness of implementation of disease control programmes and for disease surveillance (Alton et al., 2015, Fasina et al., 2015, Kaneene et al., 2006). Brucellosis is endemic in the Mediterranean Basin, Caribbean, Africa, South and Central America, Asia and

the Middle East (Adesiyun and Cazabon, 1995, Corbel, 2006, Galinska and Zagórski, 2013, Pappas et al., 2006). These areas and countries have abattoir facilities that process animals into meat products, and when *Brucella* infected animals are slaughtered, these animals can serve as a source of infection to susceptible abattoir workers, and as such, identification of risk factors that predispose abattoir workers to such zoonotic infections cannot be overemphasized. These workers may be exposed to infection through direct contact with infected animal's secretions or blood, or indirectly through the consumption of raw meat or undercooked meat and consumption of unpasteurized milk or milk products (Corbel, 2006). Aerosol dispersion of *Brucella* droplets from animal carcasses is another possible means of infection to animal or abattoir workers. *Brucellae* can persist in animal carcasses and due to working in close proximity to these carcasses; infection may be contracted through inhalation or through contact with the conjunctiva of the eyes, or a compromised skin, especially in individuals without PPE (Corbel, 2006, Galinska and Zagórski, 2013, Jones et al., 1982). Some of the risk factors that may predispose abattoir workers to infection include the number of days at work, health status of animals to be slaughtered, non-use of PPE, splash of animal secretion on the face, hand cut injuries during work, environmental hygienic measures, knowledge and attitude of the workers and their perception of zoonoses as an occupational hazard (Battelli, 2008). Many countries have reported the incidences of brucellosis in abattoir workers. A study reported a 7.9% seroprevalence among abattoir workers in Iran, and various risk factors that predisposed the workers to the infection were evaluated (Esmaeili et al., 2016). Several risk factors were tested in the Iranian study, of which 20.1% of respondents did not perceive zoonoses to be a problem at the abattoirs, while 75.3% of respondents agreed to have had animal secretion splashed onto their faces while performing their duties at the facilities (Esmaeili et al., 2016). A similar study conducted in Brazil in South America observed a 4.1% seroprevalence of brucellosis among abattoir workers (Ramos et al., 2008). In Tanzania in East Africa, 5.52% seroprevalence of brucellosis among abattoir workers was recorded (Swai and Schoonman, 2009) while in Pakistan, 21.7% seroprevalence of brucellosis was reported among abattoir workers (Mukhtar and Kokab, 2008). These studies have shown that brucellosis is an important occupational hazard among abattoir workers. Although there have been reports of brucellosis in humans in South Africa (Wojno et al., 2016), some of these incidences date back to the 18th and the 19th centuries and the infections were in farmers who contracted the disease through the consumption of unpasteurized milk (Van Den Heever et al., 1982, Van Drimmelen, 1949). To date, there are no empirical data or published reports on the risk factors, occurrence or prevalence of brucellosis in the abattoir workers in South Africa. It was therefore, important to conduct a provincial abattoir-based, evidence based-data of the risk factors that predispose abattoir workers to zoonotic infections. This current study was

therefore conducted to have evidence-based data on risk factors of brucellosis in the abattoir workers in the Gauteng province of the country.

4.3 Materials and methods

4.3.1 Study area

The study area is the Gauteng Province of South Africa (Figure 4.1). This is the smallest province accounting for 1.5% of the land area of the country and houses about 23.7% of the country's population. This province is the home to the executive arm of government and it is also the economic and financial hub of the country. It is highly urbanized and has 12 municipalities. The human population is estimated to be approximately 58 million people and the country stretches from 22°S to 35°S and from 17°E to 33°E, with a surface area of 1,219,602 km². The country has several distinct ecosystems and it is bounded by 2,798 km of coastline stretching along the South Atlantic and the Indian Oceans. In the north, its neighbouring countries are Namibia, Botswana, Zimbabwe and to the east and northwest by it is bordered by Mozambique and Swaziland. The mean annual rainfall in the country is 464 mm and the country location varies between 1087 meters to 3300 meters above sea level. The country is divided into nine provinces, with the Gauteng province hosting the executive arm of government, Cape Town the legislative arm of the government and the Free State hosting the judicial arm of the government. The estimated number of livestock in the Gauteng province as at May 2018 was cattle 246,395, sheep 92,160, goat 29,017 and pigs 156,264 (DAFF., 2018).

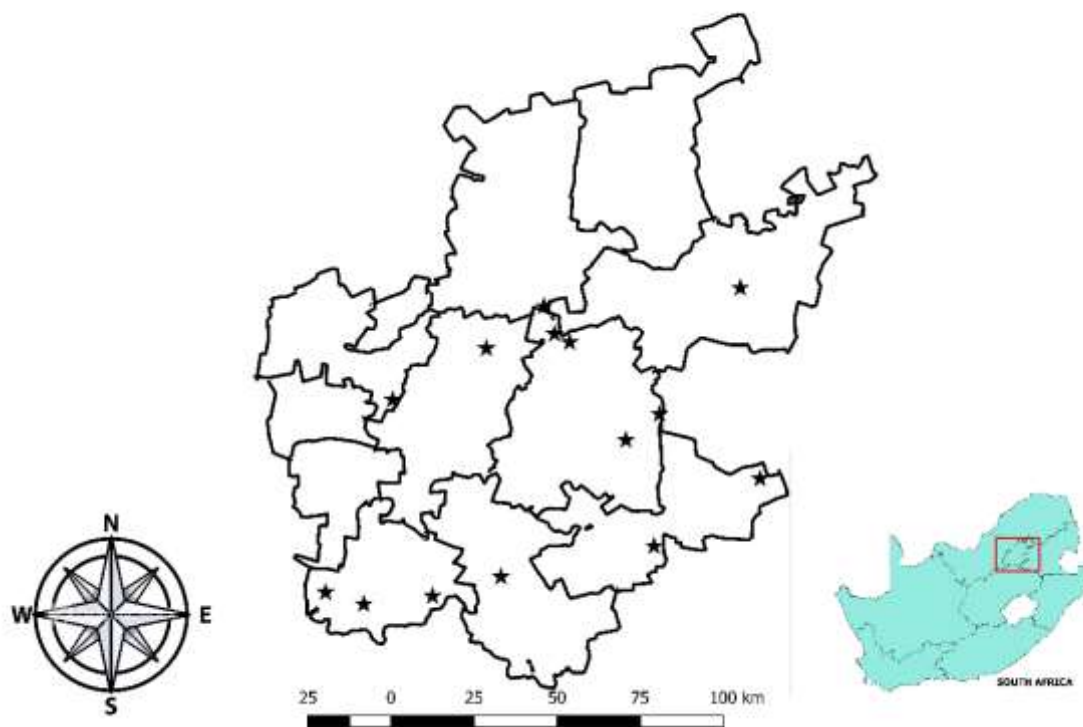


Figure 4-1: Locations of abattoirs sampled in Gauteng Province of South Africa.

4.3.2. Study design

A cross sectional study was performed in the first phase to assess the perception, determine some associated risk factors for zoonoses and educate the workers on preventive measures to infections from 2016 to 2017. Another cross-sectional study was conducted in the second phase and the study determined the seroprevalence of brucellosis among abattoir workers from 2017 to 2018 at the Gauteng province abattoirs.

4.3.3 Types of abattoirs sampled

In the first phase, fourteen operational abattoirs in Gauteng province that consented to participate in the study were purposively selected. The abattoirs selected include facilities that slaughtered cattle, sheep, goats and pigs. These abattoirs were categorized into seven high throughput (HT) and seven low throughput (LT) facilities of which some were operational as multi-species and others as mono-species. These abattoirs were included from different

municipalities of the province. All the manager/owners of the 14 abattoirs were included in the One Health stakeholder's interview.

In the second phase, six of the 14 abattoirs that were positive for brucellosis in cattle and sheep from which *B. abortus* and *B. melitensis* were isolated were included in the study, and of these six abattoirs, three were multispecies facilities where *B. melitensis* was isolated from sheep.

4.3.4 Ethical approval

Ethics approval for the study was obtained from ARC-OVI (Agricultural Research Council – Onderstepoort Veterinary Institute) Animals Ethics Committee (AEC12-16), University of Pretoria Animal Use and Care Committee (V089-16) and Department of Agriculture, Forestry and Fisheries (DAFF) Section 20 approval (Act 35 of 1984) South Africa to sample the animals at the abattoirs. Research and human Ethics approval were obtained from the University of Pretoria research Ethics committee (questioners and consent forms) and Department of Health human ethics committee (519/2017) (interview and sampling of humans).

4.3.5 Abattoir workers sample size

For the first phase, it was estimated that a maximum of 100 abattoir workers perform different duties (particularly slaughtering and dressing of carcasses) at the 14 selected abattoirs and an EpiInfo 7 statCalc for samples size and power was used to estimate the samples size at 50.0% expected frequency, with 5.0% acceptable margin of error, and with a design effect of 1 and clusters equals to 1; with a 95.0% confidence interval, a minimum sample size of 94 was expected.

For the second phase, any abattoir worker who consented in writing to participate was included in the study. A total of 103 abattoir workers, managers and meat inspectors consented to participate in the study. All abattoir workers who met the eligibility criteria were interviewed. A questionnaire which extracted demographic data and duties performed at the abattoir was administered to each participant using interpreters among the research team members, when necessary (Appendix 2). The sampling was conducted based on the type of duties performed at the facility which included whether the abattoir worker was a butcher ('slaughter man'), inspector, transporter and others (a description of any other type of work being done at the abattoir).

4.3.6 Study population

The inclusion criteria for the study population was all the managers who submitted signed written consent forms and their abattoir workers, who consented verbally were included in the study on the day of a visit to the abattoirs. All abattoir managers who signed the consent forms as stakeholders gave permission to conduct the study in their facilities. The consenting managers and abattoir workers were interviewed by the study team.

4.3.7 Sampling method and recruitment

Sample size for human workers in the abattoirs was determined according to Thrusfield (2007): $n_o = t^2(p)(1-p)/d^2$ where $t=1.96$, p =prevalence, d =precision at a type 1 error of 0.05, n_o =estimated sample size. Since there are no current data on the seroprevalence of human brucellosis in abattoir workers in Gauteng province or South Africa at large, the prevalence was therefore assumed to be 50% (0.5). For the current study, the minimum sample size was therefore determined using the following criteria; $P=0.5$ and a precision of 10%. A minimum estimated sample size, n_o =was then $(1.96)^2 \times (0.5) \times (1 - 0.5) / (0.01)^2 = 3.84 \times 0.25 / 0.0036 = 0.96 / 0.001 = 96$. For the second phase study, a total of 103 humans were sampled.

4.3.8 Pre-testing of questionnaire and data collection

A structured questionnaire was constructed with three parts on Microsoft Word Office 2007 and this was tested on five abattoir workers at the first abattoir visited in Pretoria, Gauteng Province, South Africa, and necessary adjustments were conducted. The first part of the questionnaire extracted demographic data on the worker comprising the age, sex, marital status, number of years at work, job description and animal slaughter section stationed. The second part consisted of close-ended questions about attitudinal risky practices during work, such as the use of protective clothing, gloves, eye goggles, mask, eating of raw meat, and splash of blood or fluid into the eyes during work. The last part consisted of questions regarding their perception of the probability of contracting zoonoses at the abattoir and their attitude towards seeking medical help whenever they felt the symptoms of illness (Appendix 1).

The managers/owner's questionnaire included questions on the number of staff at the facility, knowledge of zoonoses, education of workers, provision of PPE, enforcement of wearing PPE, workers engagement in risky practices during work, previous diagnosis of workers with zoonoses and testing of workers for zoonoses at the abattoirs (Appendix 3).

During the second phase conducted 12 months after the initial phase, six abattoirs were selected based on the detection of brucellosis positive cattle and sheep describe in Chapter

5., Information retention about zoonoses conveyed to 103 abattoir workers during the first phase was assessed. A structured questionnaire in Microsoft Office Word 2007 was used to interview the contending respondents (Appendix 2).

4.3.9 Data collection

All abattoir workers who met the eligibility criteria were interviewed using the structured questionnaire. The interview was conducted by the research team which had an interpreter who translated the questions into the preferred language of the respondent (Appendix 1).

4.3.10 Collection of blood samples

A qualified phlebotomist was recruited to collect 5 ml of blood from each consenting worker following established procedure during and post-collection of blood samples. One hundred and three un-clotted blood samples were collected in vacutainer tubes without any anti-coagulant to harvest sera for the serological assays.

4.3.11 Serology tests conducted (RBT, BrucellaCap and IgG ELISA)

All the serological tests were conducted at the special pathogen laboratory of the National Institute for Communicable Diseases (NICD), South Africa.

Rose Bengal test (RBT): The commercial IDEXX *Brucella* antigen (Switzerland) stained with Rose Bengal (30 µl) was mixed with an equal volume of serum sample and the mixture was agitated gently for four minutes at room temperature on a Shaker. Agglutination was read after four minutes and any visible agglutination was regarded as positive for brucellosis, with positive and negative controls supplied by the kit. The diagnostic sensitivity and specificity for the RBT were assumed to be 100% and 75%, respectively, based on previous validation studies (Stemshorn et al., 1985).

BRUCELLACAPT® (Vircell Microbiologist, Spain): This test is a single-step immunocapture assay for the detection of total anti-*Brucella* antibodies (mainly IgM) and was done according to the manufacturer's instructions, with the positive and negative controls included in the kit.

BRUCELLA ELISA IgG (Vircell Microbiologist, Spain). This ELISA test was performed according to the manufacturer's instructions. Human sera were added to microtitre plates with antigen adsorbed on the polystyrene surface. Unbound immunoglobulins were washed off as the enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After another wash step, bound conjugate was developed with the aid of a substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding acid stopping solution. The ELISA washer model used was ELx50/8 by BioTek, the ELISA

reader model used was ELx800 by BioTek and the software program used to read and interpret the results was GEN 5 Software package by BioTek. The positive and negative controls were supplied by the kit.

4.3.12 Statistical analysis

Data collected were managed using the Epidata software 2010 version. The data were entered into the software by two individuals independently to check for consistency and other forms of data entry errors. The R software (RCoreTeam, 2013) was used to analyze the data and to conduct descriptive analysis, Epi-Info 7 version 10 was used for bivariate analysis of odd ratios, chi square test and for plotting of charts. These were conducted for the measurement of the direction and the strength of association between two categorical variables.

In the second phase, all variables statistically significant with $P \leq 0.3$ as the inclusion criteria were used from the bivariate analysis in the logistic regression model (Dohoo et al., 2009). Hosmer-Lemeshow χ^2 was used as a goodness of fit test, Small values with large p-values indicate a good fit to the data. STATA package was used for regression analysis (StataCorp, 2007). Epi-Info software 7 version 10 was used for univariate and bivariate analysis of risk factors and for plotting of charts. QGIS software 2.14.0-Essen was used to construct the map.

4.3.13 Stakeholders for zoonoses awareness campaign and education materials

Stakeholders to help with the awareness dissemination and propagation programme were selected using stakeholder matrix and analysis which uses the strengths, weaknesses, opportunities and threats (SWOT) analysis model, the stakeholders included the principal researcher, University students, abattoir managers, abattoir inspectors, abattoir workers, state veterinarians and environmental monitors. The stakeholder matrix was used to evaluate each stakeholder assessing the basic characteristics of the stakeholder in terms of their interests and how they are affected by the problem of zoonoses coupled with their contributions to the project, and their capacity and motivation to bring about change and possible action to address their interests (Bryson, 2004).

A SWOT analysis was used as a tool for general analysis to assess how the abattoir organization, the state public health sector and institutions of higher learning (the University of Pretoria, Tshwane University of Technology, South Africa) might address the risk factors as problems or challenges. The internal strengths and weaknesses of the abattoirs and the state public health institutions with regards to the external opportunities and threats that they face were analyzed. Information extracted from this analysis was dependent on the stakeholders involved and was managed based on the quality of information derived. The situation was

analyzed by looking for ways by which the stakeholders' strengths could be built on to overcome identified weaknesses, and how the opportunities could be taken to minimize threats; and finally a strategy for making improvements was formulated and subsequently developed by discussion (Renault, 2013). A multidisciplinary approach was then used to produce education materials. The multidisciplinary stakeholders involved were students from disciplines such as veterinary medicine, public health, digital media and printing profession, language profession, eco-tourism, medical profession all from the University of Pretoria and Tshwane University of Technology, South Africa.

After interviewing respondents at each abattoir, they were all subjected to sensitization and awareness about zoonotic diseases, focusing on brucellosis, tuberculosis, leptospirosis, toxoplasmosis, Q-fever and cysticercosis (Appendix 4).

4.4 Results

4.4.1 Initial phase: Analysis of data from the abattoir managers/owners

The managers were grouped into two classes comprising seven from the LT abattoirs with a mean staff number of 17 (range: 9 to 38), and seven HT abattoir managers, with a mean staff number of 158 (range 28 to 360). Of these 14 managers, 71.4% (10/14) indicated they had information about most of the zoonotic diseases (brucellosis, tuberculosis, leptospirosis, cysticercosis, toxoplasmosis and Q-fever) in the questionnaire. However, 28.6% (4/14) had no knowledge about Q-fever but had knowledge on the other zoonotic diseases in questionnaire. All the 14 managers indicated that they provided education to their workers about zoonoses at least once a year and always provided PPE to the workers and enforced PPE usage during work. None had knowledge that abattoir workers had been diagnosed with any of the zoonoses in the questionnaire for at least three years prior to our interview. Of the 14 managers, 4 (28.6%) confirmed that some of their workers sometimes engaged in risky practices such as refusal to wear PPE and refusal to comply with hygienic measures such as regular hand washing when on duty. Of the abattoirs that engaged in risky practices, 75.0% (3/4) belonged to the HT facilities while 25.0% (1/4) were LT facilities. Five (35.7%) of the 14 managers indicated that workers were not tested for zoonoses, while 64.3% (9/14) indicated regular testing of their workers for zoonoses. The abattoirs that did not test their workers for zoonoses, 40.0% (2/5) were HT facilities while 60.0% (3/5) were LT. The 14 consenting abattoir managers were also stakeholders in the zoonoses awareness program and participated in the study. From the SWOT analysis, their interest was to trained workers on

zoonoses, their strength was access to the abattoir facilities and the opportunity of the researchers to contact their staff.

4.4.2 Initial phase: Analysis of demographic risk factors of abattoir workers

Of the 143 respondents, 22.0% (31/143) were females and 78.0% (112/143) were males (Figure 4.2). The age categories are presented in Figure 4.3, of which 41.0% (59/143), 56.0% (80/143) and 3.0% (6/143) belonged to the age categories of 16-30 years, 31-60 and 61 years and above respectively. It was observed that 93.0% (133/143) of the respondents worked in all animal processing sections (cattle, sheep, goat, and pigs) of the abattoir, while 7.0% (10/143) worked only in pig processing section of the mono-species abattoir. All the abattoir facilities visited had a water supply for washing hands, rinsing carcasses and cleaning of the facility, 64.3% (92/143) of the abattoirs water supply was from the municipality and 35.7% (51/143) was supplied through borehole constructed by the facilities.

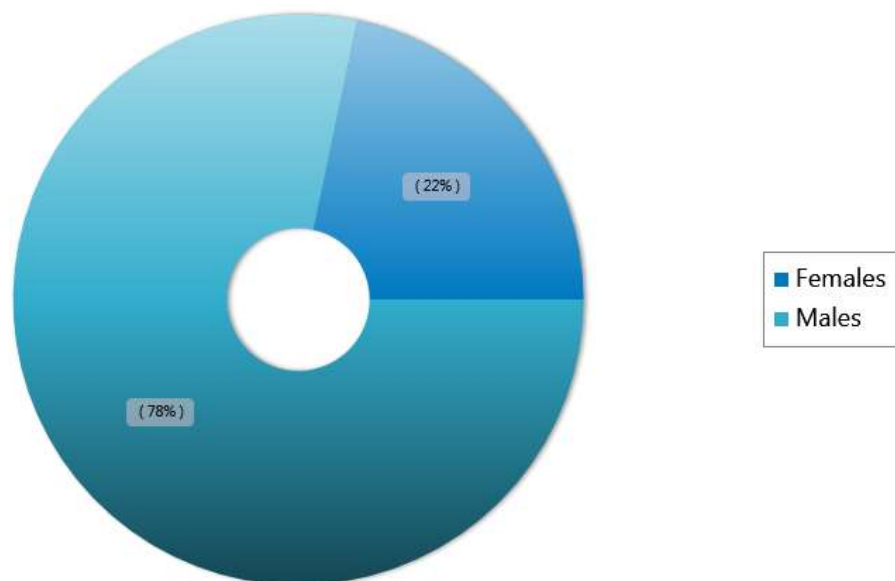


Figure 4-2: Distribution of respondents according to gender.

Of the 143 respondents, 34.3% (49/143) have worked in the facilities for one year or less, of this group, 55.1% (27/49) were in the age category 16-30 years, and 44.9% (22/49) belonged to the age category 31-60 years, none was above 60 years. Seventeen (11.9%) of the 143 workers have worked at the facilities for 2 years, of this group, 35.3% (6/17) were in the 16-30 years category while 64.7% (11/17) were of the age category 31-60 years. It was observed that 53.8% (77/143) have worked at the facilities for three years and above, of this group,

33.8% (26/77) were of the age category 16-30 years, while 61.0% (47/77) were of the age category 31-60 years, and 5.2% (4/77) were of the age category 61 years and above.

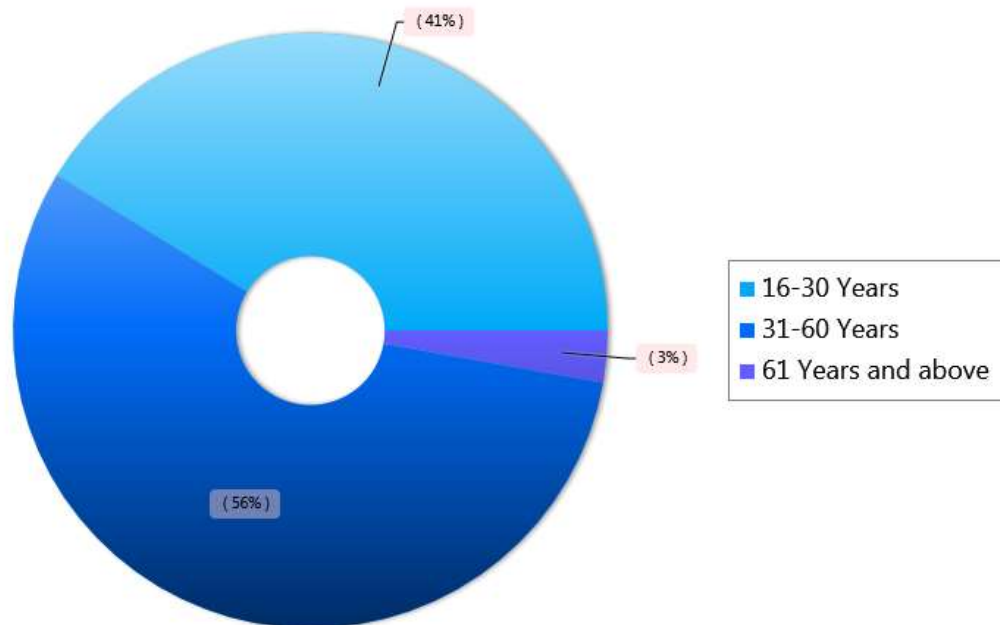


Figure 4-3: Distribution of respondents according to the age categories.

A clear majority, 125 (87.4%) of the 143 respondents worked in the slaughter man or butcher category, of which there were more male than female workers and they belonged more to the 31- 60 year age category compared to others. The inspector category was 10.5% (15/143), of which the males were more compared to females and the age category 31 - 60 years were more represented than others. For the categories of manager and transporter, only one person each was represented respectively, and they belonged to the age categories of 31 – 60 years and 16 – 31 years respectively. For the cleaner category only one female was represented, and she was under the age category of 31 – 60 years (Table 4.1).

Table 4.1: Distribution of the abattoir workers (n=143) by the types of duty performed stratified by gender and age category in Gauteng province participating in the first phase of this study.

Duty	Total 143	Gender (%)		Age Category (%)		
		Female	Male	16-30 yr*	31-60yr	61yr.and above
Slaughter man	125	25 (20.0)	100 (80.0)	56 (44.8)	65 (52.0)	4 (3.2)
Inspector	15	5 (33.3)	10 (66.7)	2 (13.3)	13 (86.7)	0 (0.0)
Transporter	1	0 (0.0)	1 (100)	1 (100)	0 (0.0)	0 (0.0)
Manager	1	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0 (0.0)
Cleaner	1	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)

*Yr- years, %- percentage

A percentage of, 37.1% (53/143) were married and of this 7.5% (4/53) were female and 92.5% (49/53) were male workers (Figure 4.4). The proportion of the single group was 58.7% (84/143), of which 31.0% (26/84) were female and 69.0% (58/84) were male workers (Figure 4.4). The proportion of the divorced group was 4.5% (6/143) of which 16.7% (1/6) were females and 83.3% (5/6) were males (Figure 4.4).

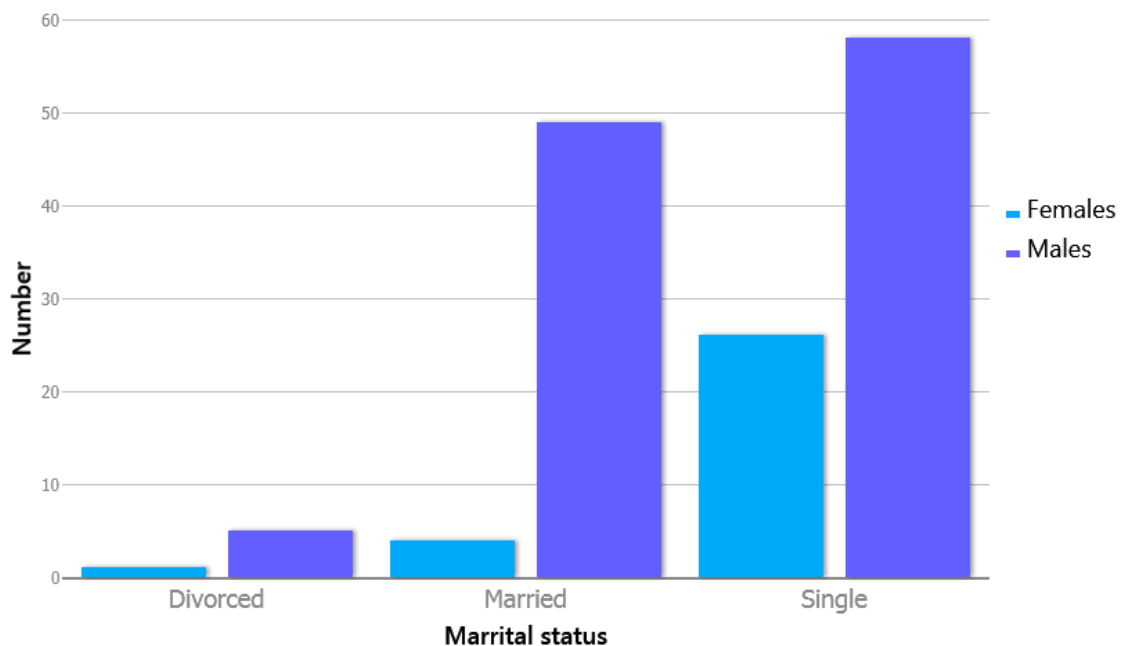


Figure 4-4: Distribution of respondents according to marital status stratified by gender.

Among 53 married respondents, stratified by age; 18.7% (10/53) were in the age 16 – 30 years category, 75.5% (40/53) were of age 31 – 60 years and 5.7% (3/53) were of the age category 61 years and above (Figure 4.5). Of the 84 in the single group, 60.0% (47/84) were of the age category 16 – 30 years, 42.8% (36/84) were of the age category 31 – 60 years, and 1.2% (1/84) were of the age group 61 years and above. Of the six in the divorced group, 33.3% (2/6) were of the age category 16 – 30 years and 66.7% (4/6) were of the age category 31 – 60 years.

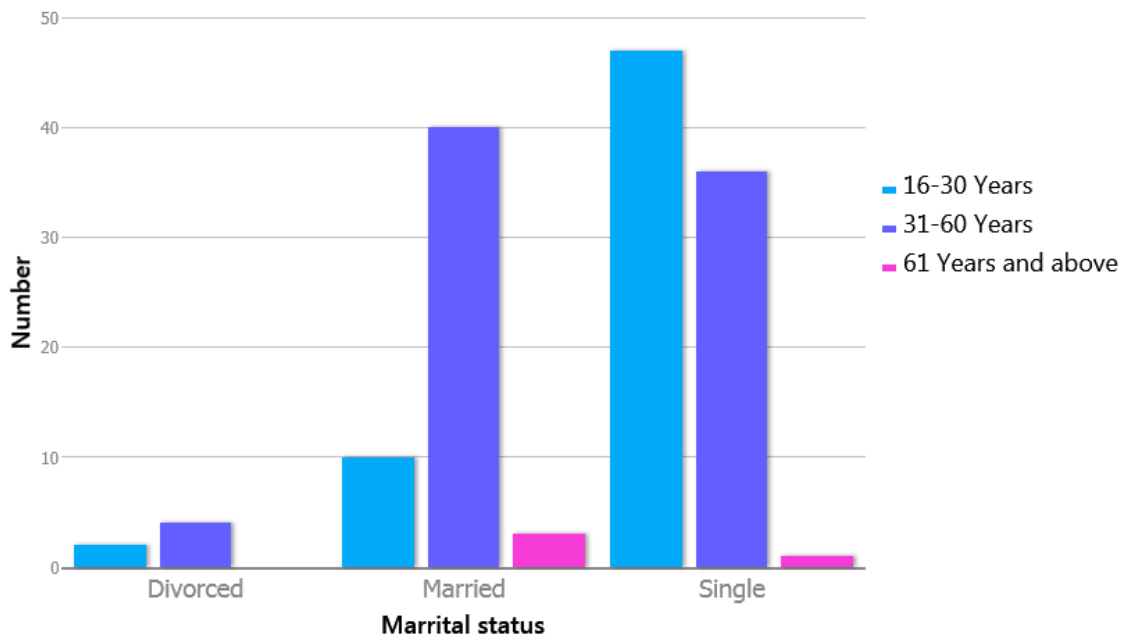


Figure 4-5: Distribution of respondents according to the marital status stratified by age categories.

4.4.3 Initial phase: Descriptive analysis of attitudinal, perceptual and hygienic risk factors

A proportion of 6.3% (9/143) agreed to eating raw meat while working at the abattoirs, 1.4% (2/143) agreed to stealing and consuming condemned meat; 84.0% (120/143) had hand cut injuries at least once while working at the abattoirs; 91.0% (130/143) experienced splash of blood or fluid on their faces while working; 69.9% (100/143) agreed to not using facial masks; 58.0% (83/143) agreed to not using hand gloves; 0.69% (1/143) agreed to not washing their hands regularly; 0.69% (1/143) did not regularly use PPE (coveralls, aprons, hair nets, beard nets) while working at the abattoirs; 0.69% (1/143) agreed to not using gumboots at the abattoirs; 90.2% (129/143) agreed to not using facial protective goggles; 38.5% (55/143) agreed to eating breakfast meals while having their dirty or stained abattoir uniforms (PPE) on; 67.1% (96/143) confirmed to have experienced symptoms of febrile illness (fever and flu) at least once while performing their duties at the abattoirs; 88.1% (126/143) agreed to not seeking medical help whenever they felt symptoms of illness and 37.1% (53/143) believed and had the perception that they could not acquire diseases from animals while working at the abattoirs.

Table 4.2: Bivariate analyses of risk factors associated with the probability of infection among female, male and age categories of respondents from all 14 abattoirs in Gauteng province.

Risk factor/Gender/Age category	No. (%) affected	OR (95% CI)	Chi (X ²)	P - value
Hand cut injuries	120			
Female	30 (25.0)	0.14 (0.02-1.05)	3.7	0.05
Male	90 (75.0)			
16-30 years	51 (42.5)			
31-60 years	65 (54.2)	-	1.47	0.48
>60 years	4 (3.3)			
Splash of blood on face	130			
Female	29 (22.3)	0.63 (0.13-3.02)	0.05	0.82
Male	101 (77.7)			
16-30 years	58 (44.6)			
31-60 years	68 (52.3)	-	7.68	0.02
>60 years	4 (3.1)			
Facial mask –do not use	100			
Female	23(23.0)	0.79 (0.31-1.88)	0.13	0.72
Male	77 (77.0)			
16-30 years	47 (47.0)			
31-60 years	50 (50.0)	-	4.81	0.09
>60 years	3 (3.0)			
Hand gloves –do not use	83			
Female	18 (21.7)	0.10 (0.45-2.24)	0.0	1.00
Male	65 (78.3)			
16-30 years	40 (48.0)			
31-60 years	40 (48.0)	-	4.90	0,09
>60 years	3 (4.0)			
Facial goggles-do not use	129			
Female	29 (22.5)	0.57 (0.12-2.71)	0.13	0.73
Male	100 (77.5)			
16-30 years	56 (43.4)			
31-60 years	69 (53.5)	-	3.33	0.19
>60 years	4 (3.1)			
Eating with protective cloth on	88	0.59 (0.25-1.39)-		
Female	22 (25.0)		1.02	0.31
Male	66 (75.0)			
16-30 years	39 (44.3)			
31-60 years	47 (53.4)		1.01	0.60
>60 years	2 (2.3)			
Experience of febrile illness	47	0.43 (0.19-0.96)		
Female	15 (32.0)	-	3.47	0.06
Male	32 (68.1)			
16-30 years	18 (38.3)			
31-60 years	28 (59.6)		0.42	0.81
>60 years	1 (2.1)			
Medical help seeking attitude	126	0.45 (0.09-2.06)		
Female	29 (23.0)	-	0.55	0.46
Male	97 (77.0)			
16-30 years	52 (41.3)			
31-60 years	71 (56.3)		0.67	0.71
>60 years	3 (2.4)			
Perception of no infection at abattoir	53			
Female	9 (17.0)	0.63 (0.27-1.59)	0.70	0.40
Male	44 (83.0)			
16-30 years	25 (47.2)			
31-60 years	27 (50.9)	-	1.34	0.51
>60 years	1 (1.9)			

OR-odds ratio, %-percentage, No.-number

Table 4.2 describes the odds ratio related to risk factors stratified by gender and the age categories among the respondents. The odds of female workers having hand cut injuries are 0.14 times the odds of male workers having hand cut injuries. This was statistically significant (P=0.05). The splash of blood on the faces of the respondents among the age categories was statistically significant (P=0.02).

Table 4.3: Risk factors among abattoirs workers in Gauteng province (n=143) stratified by abattoir throughput (low and high) in which they operated.

Risk factors / High and Low throughput abattoirs	No. affected (%)	OR (95% CI)	Chi ²	P - value
Hand cut injuries High throughput Low throughput	120 67 (55.8) 53 (44.2)	0.67 (0.27-1.71)	0.36	0.55
Splash of blood on face High throughput Low throughput	130 74 (56.9) 56 (43.1)	0.83 (0.26-2.66)	0.0	0.98
Facial mask – do not use High throughput Low throughput	100 62 (62.0) 38 (38.0)	0.53 (0.23-1.09)	2.34	0.13
Hand gloves – do not use High throughput Low throughput	83 49 (59.0) 34 (41.0)	0.85 (0.43-1.66)	0.10	0.76
Facial goggles-do not use High throughput Low throughput	129 71 (55.0) 58 (45.0)	0.33 (0.09-1.25)	1.98	0.16
Eating with PPE on High throughput Low throughput	88 58 (65.9) 30 (34.1)	0.40 (0.20-0.79)	5.98	0.01
Experience of febrile illness High throughput Low throughput	47 20 (42.5) 27 (57.4)	0.41 (0.20-0.83)	5.39	0.02
Medical help seeking attitude High throughput Low throughput	126 76 (60.3) 50 (39.7)	0.36 (0.12-1.03)	2.88	0.08
Perception of no infection at abattoir High throughput Low throughput	53 31 (58.5) 22 (41.5)	0.93 (0.47-1.85)	0.0	0.97

Based on the type of abattoirs, the respondents were divided into two categories, 57.3% (82/143) worked in the high throughput abattoirs and 42.7% (61/143) worked in the low throughput abattoirs. The important result from Table 4.3 describes the odds of respondents in the HT abattoirs eating with their abattoir PPE on are 0.40 the odds of respondents in LT abattoirs eating with abattoir PPE on. This was statistically significant (P=0.01). The odds of respondents in HT abattoirs experiencing symptoms of febrile illness are 0.41 times the odds of respondents in LT abattoirs experiencing symptoms of febrile illness. This was statistically significant (P=0.02).

Among the 120 respondents with hand cut injuries, 33.3% (40/120), 12.5% (15/120) and 54.2% (65/120) of hand cut injuries was amongst those working for one year or less, two years and three years and above, respectively.

Experiences of splash of animal blood or fluid on the faces while performing their duties, varied from 33.1% (43/130) among those who had worked for one year or less, to 13.1% (17/130) among those had worked for two years, while 53.8% (70/130) was among those who had worked for three years and above.

For defaulters who did not use face masks, 35.0% (35/100) had worked for one year or less, 11.0% (11/100) had worked for two years, while 54.0% (54/100) had worked for three years and above.

For those that did not use hand gloves during duty, 37.3% (31/83) have worked for one year or less; 8.4% (7/83) have worked for two years, while 54.2% (45/83) have worked for three years.

Respondents who did not use facial goggles while working at the abattoirs, 35.6% (46/129) have worked for one year or less, 13.2% (17/129) have worked for two years, while 51.2% (66/129) who have worked for three years and above and all agreed to not using facial masks while working at the abattoirs.

Those who ate breakfast with their personal protective equipment (PPE) on, 38.6% (34/88) had worked for one year or less, 10.2% (9/88) had worked for two years, while 51.1% (45/88) worked for three years and above.

For the respondent that experienced symptoms of febrile illness while on duty, 23.4% (11/47) have worked for one year or less, 12.8% (6/47) had worked for two years, while 63.8% (30/47) had worked for three years and above.

Of the respondents who did not seek medical help when they experienced symptoms of febrile illness while working at the abattoirs or at home, 38.1% (48/126) have worked for one year or less, 12.7% (16/126) had worked for two years, while 49.2% (62/126) had worked for three years and above.

Among the respondents who could not perceive that they can contract zoonoses from animals while working at the abattoirs, 37.7% (20/53) had worked for one year or less, 7.5% (4/53) had worked for two years and 54.7% (29/53) had worked for more than three years.

Table 4.4: Table showing the analysis of risk factors in relation to the duration of years of work and within the age category of the respondents.

Risk Factors	Total No. Affected	Years at work (No.)			Age Category (No.)		
		1 yr (49) (%)	2 yr. (17) (%)	3 yr./above (77) (%)	16-30 yr. (59) (%)	31-60 yr. (80) (%)	60 yr./above (4) (%)
Hand cut injuries	120	40 (81.6)	15 (88.2)	65 (84.4)	51 (86.4)	65 (81.2)	4 (100)
Splash of blood on face	130	43 (87.7)	17 (100)	70 (91.0)	58 (98.3)	68 (85.0)	4 (100)
Facial mask –do not use	100	35 (71.4)	11 (64.7)	54 (70.1)	47 (79.7)	50 (62.5)	3 (75.0)
Hand gloves –do not use	83	31 (63.2)	7 (41.2)	45 (58.4)	40 (67.8)	40 (50.0)	3 (75.0)
Facial goggles-do not use	129	46 (93.9)	17 (100)	66 (85.7)	56 (95.0)	69 (86.2)	4 (100)
Eating with PPE on	88	34 (69.4)	9 (53.0)	45 (58.4)	39 (66.1)	47 (58.7)	2 (50.0)
Experienced of febrile illness	47	11 (22.4)	6 (35.3)	30 (39.0)	18 (30.5)	28 (35.0)	1 (25.0)
Medical help seeking attitude	126	48 (98.0)	16 (94.1)	62 (80.5)	52 (88.1)	71 (88.5)	3 (75.0)
Perception of no infection at abattoir	53	20 (40.8)	4 (23.5)	29 (37.7)	25 (42.4)	27 (33.7)	1 (25.0)

*No.-Number, PPE-personal protective equipment, %-percentage

The explanatory risk factors measured varied between the years at work and the age categories (Table 4.4).

4.4.4 Second phase analysis of follow up interview of abattoir workers on zoonoses awareness campaign

Of the 103 respondents tested, the frequency and distribution of females were 16 (15.5%, 95%CI=9.15-24.0) and 87 (84.5%, 95%CI=76.0-90.8) were for males. The mean age of the respondents was 36.4 (range 18-60). The mean age for females was 32.6 (range 23-49) and the mean age for males was 37.1 (range 18-60). Table 4.5 reveals the feedback on the knowledge of the zoonoses sensitization campaign conducted during the first round.

Table 4.5: The respondent's response on the knowledge of zoonoses stratified by gender in abattoirs in Gauteng province.

Type of Zoonoses	Response (%)		Odds ratio Gender OR (95% CI)	Association Gender	
	Yes (%)	No (%)		Chi (X) ²	P-value
Brucellosis	27 (26.2)	76 (73.8)	1.34 (0.42-4.30)	0.0	0.85
Female	5 (4.8)	11 (10.7)			
Male	22 (21.4)	65 (63.1)			
Cysticercosis	8 (7.8)	95 (92.2)	****	0.56	0.45
Female	0 (0.0)	16 (15.5)			
Male	8 (7.8)	79 (76.7)			
Leptospirosis	16 (15.5)	87 (84.5)	1.31 (0.33-5.26)	0.0	0.10
Female	3 (2.9)	13 (12.6)			
Male	13 (12.6)	74 (72.0)			
Q-fever	12 (11.7)	91 (88.3)	2.00 (0.48-8.38)	0.29	0.39
Female	3 (2.9)	13 (12.6)			
Male	9 (8.7)	78 (75.7)			
Tuberculosis	91 (88.3)	12 (11.7)	2.17 (0.26-18.10)	0.10	0.76
Female	15 (14.6)	1 (1.0)			
Male	76 (73.8)	11 (10.7)			
Toxoplasmosis	4 (3.9)	99 (96.1)	6.07 (0.79-46.69)	1.53	0.22
Female	2 (2.0)	14 (13.6)			
Male	2 (2.0)	85 (82.5)			

OR-odds ratio, %-percentage, ****-undefined

The odds of females recalling the knowledge of zoonoses were higher than males (Table 4.5).

4.4.5 Analysis of demographic data from abattoir workers for brucellosis tests in the second phase

The distribution of respondents according to age category for 18-30 year old were 32 (31.1%, 95%CI=22.3-40.9) and for age category 31-60 year old was 71 (68.9%, 95%CI=59.1-77.7).

According to the respondents' marital status, the frequency and distribution of married respondents were 42 (40.8%, 95%CI=31.2-50.9) and for single respondents was 61 (58.2%, 95%CI=49.1-68.8). The frequency and distribution of respondents according to marital status stratified by age as illustrated in Figure 4.6, for age category 18-30 years and married was 8 (25.0%, 95% CI=11.5-43.4), while for 18-30 years and single was 24 (75.0%, 95%CI=56.6-

88.5). For age category 31-60 years and married the frequency and distribution was 34 (47.9%, 95%CI=35.9-60.1), while for age category 31-60 years and single, the frequency and distribution was 37 (52.1%, 95%CI=39.9-64.1).

The mean age for married female and single female workers was 33 (range 25-49) and 32.4 (range 23-48) respectively. The mean age for married male and single male workers was 41.9 (range 28-60), and the mean age for single males was 33.7 (range 18-57).

The frequency and distribution of the respondents according to the type of duties performed at the abattoirs is illustrated in Figure 4.7, and it was observed as follows; Slaughter man (butchers) = 68 (66.0%, 95%CI=56.0-75.1), Inspectors 14 = (14.0%, 95%CI=7.63-21.7), Cleaners = 8 (8.0%, 95%CI=3.41-14.7), Washing offal = 7 (7.0%, 95%CI=2.78-13.5), Manager = 3 (3.0%, 95%CI=0.60-8.28), Transporter = 2 (2.0%, 95%CI=0.24-6.84) and Hides and skin = 1 (1.0%, 95%CI=0.02-5.29).

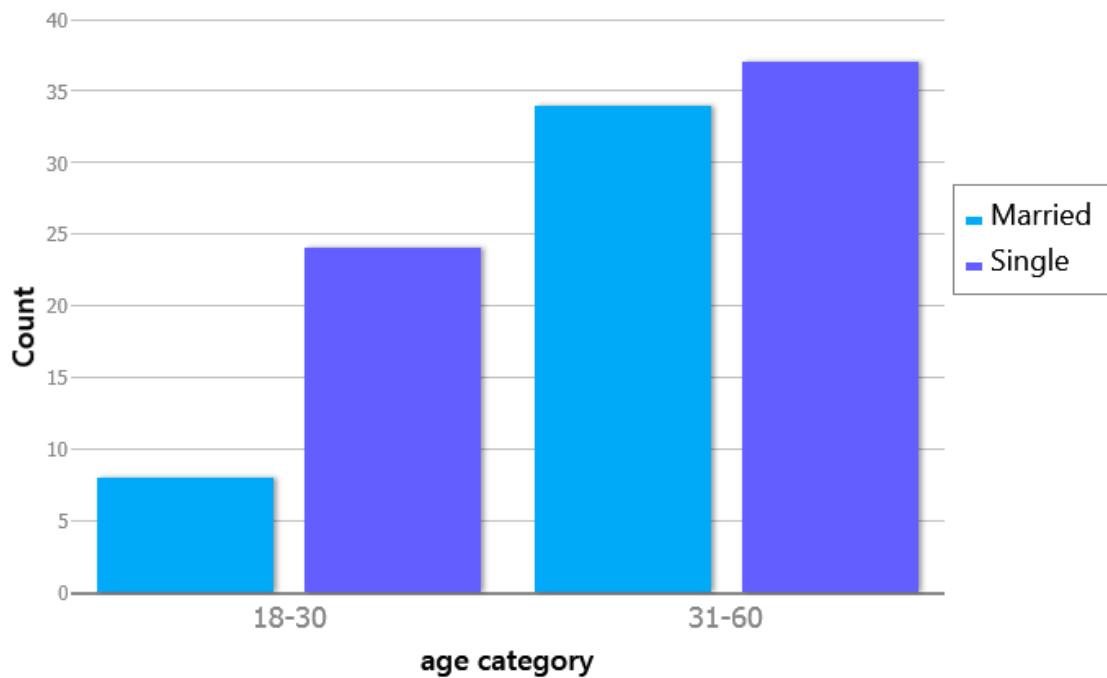


Figure 4-6: Distribution of married respondents stratified by their age categories.

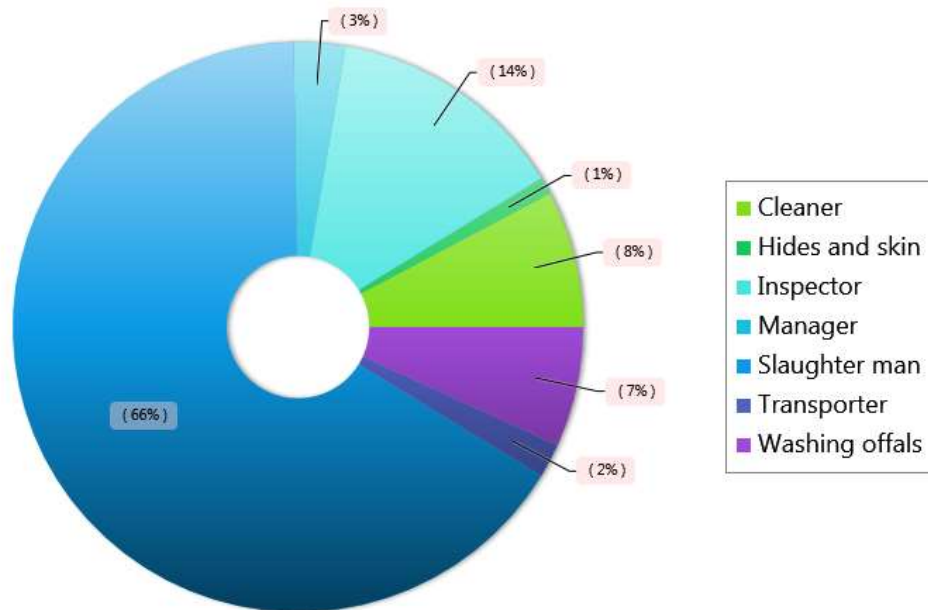


Figure 4-7: Distribution of respondents (n=103) according to duties performed at the abattoirs.

Of the 103 respondents, the frequency of distribution according to the number of years worked at the abattoir is as follows; work duration of 1 year, 21 (20.3%, 95%CI=13.1-29.5); work duration of 2 years 17 (16.5%, 95%CI=9.92-25.1); and work duration of 3 years and above, 65 (63.1%, 95%CI=53.0-72.4).

According to the number of days at work weekly, the frequency and distribution of those who worked for 5 to 7 days per week was 98 (95.2%, 95%CI=89.0-98.4) and those who worked for 3 to 4 days was 2 (1.94%, 95%CI=0.24-6.84) and those who worked for 1 to 2 days was 3 (2.91%, 95%CI=0.60-8.28).

4.4.6 Analysis of seropositivity among the abattoir workers

Of the 103 abattoir workers' serum samples tested with combined serological tests, the overall distribution and seroprevalence for *Brucella* spp. infection or exposure was 21 (20.4%, 95%CI=13.1-29.5). With RBT the distribution and seroprevalence were 13 (12.6%, 95%CI=6.89-20.6), with BrucellaCap the distribution and seroprevalence was 9 (8.74%, 95%CI=4.07-15.9) and with IgG-ELISA assay the distribution and seroprevalence were 18 (17.5%, 95%CI=10.7-26.2).

The overlap and non-overlap of serological results for the three tests conducted is illustrated in Table 4.6. The distribution and seropositivity with RBT and BrucellaCap was 7 (6.80%, 95%CI: 2.78-13.5), for RBT and IgG-ELISA was 10 (9.71%, 95%CI: 4.75-17.1), for

BrucellaCap and IgG-ELISA was 9 (8.74%, 95%CI: 4.07-15.9) and for RBT, BrucellaCap and IgG-ELISA was 7 (6.80%, 95%CI: 2.78-13.5).

Table 4.6: Overlap and non-overlap of serological results for RBT, BrucellaCap and IgG-ELISA testing of abattoir workers at Gauteng province.

Test	No. of sera tested	No. (%) positive	95% CI
RBT	103	13 (12.6)	6.89-20.62
BrucellaCap	103	9 (8.7)	4.07-15.94
IgG-ELISA	103	18 (17.5)	10.70-26.21
RBT/BrucellaCap	103	7 (6.8)	2.78-13.50
RBT/IgG-ELISA	103	10 (9.7)	4.75-17.13
BrucellaCap/IgG-ELISA	103	9 (8.7)	4.07-15.94
RBT/BrucellaCap/IgG-ELISA	103	7 (6.8)	2.78-13.50

From the 21 seropositive samples according to the combined serological tests, the frequency and distribution of seropositivity varied by gender from one case (4.76%. 95%CI=0.12-23.8) among females to 20 (95.2%, 95%CI=76.2-99.8) among males (Figure 4.8).

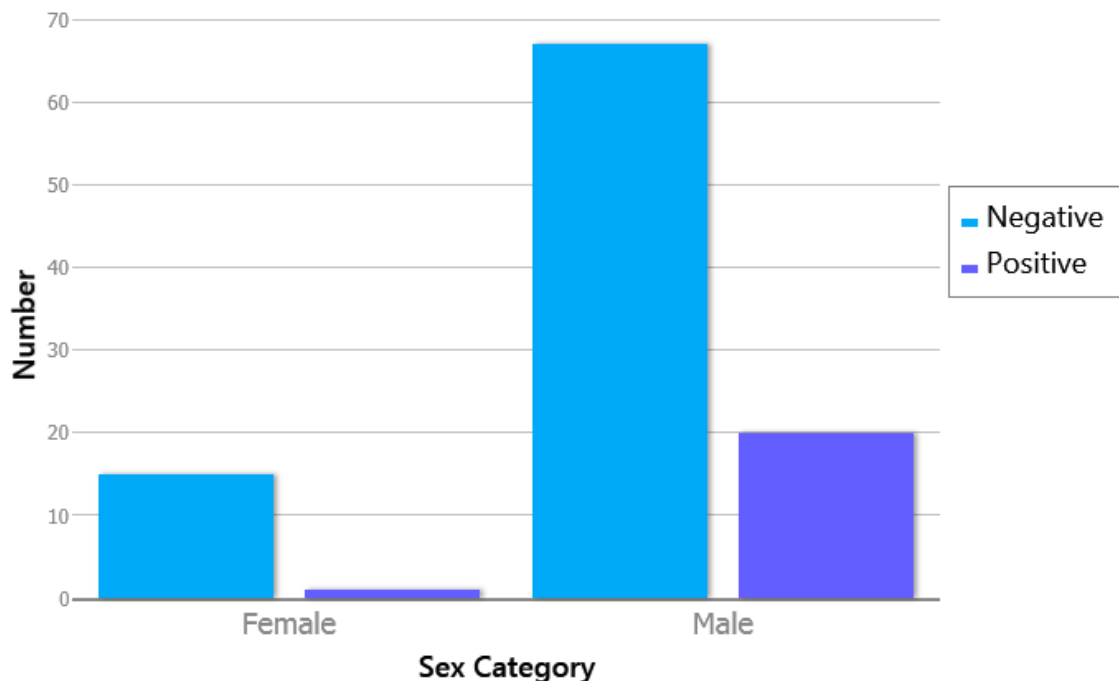


Figure 4-8: Distribution of seropositivity by gender.

The seroprevalence rate among the female and male workers during the study period was 6.25% (1/16) and 22.9% (20/87), respectively. The difference was statistically significant ($P = 0.00$) and the odds of females becoming infected with *Brucella* spp. are 0.21 times the odds of males becoming infected (Table 4.7).

Among the 21 seropositive workers, the frequency and distribution of seropositivity is illustrated in Figure 4.9. The age group (18-30 years) accounted for 5 (23.8%, 95%CI=8.22-

47.2) and the age group (31-60 years) accounted for 16 (76.2%, 95%CI=52.3-91.8). The difference in seropositivity between both groups was statistically significant ($P=0.002$).

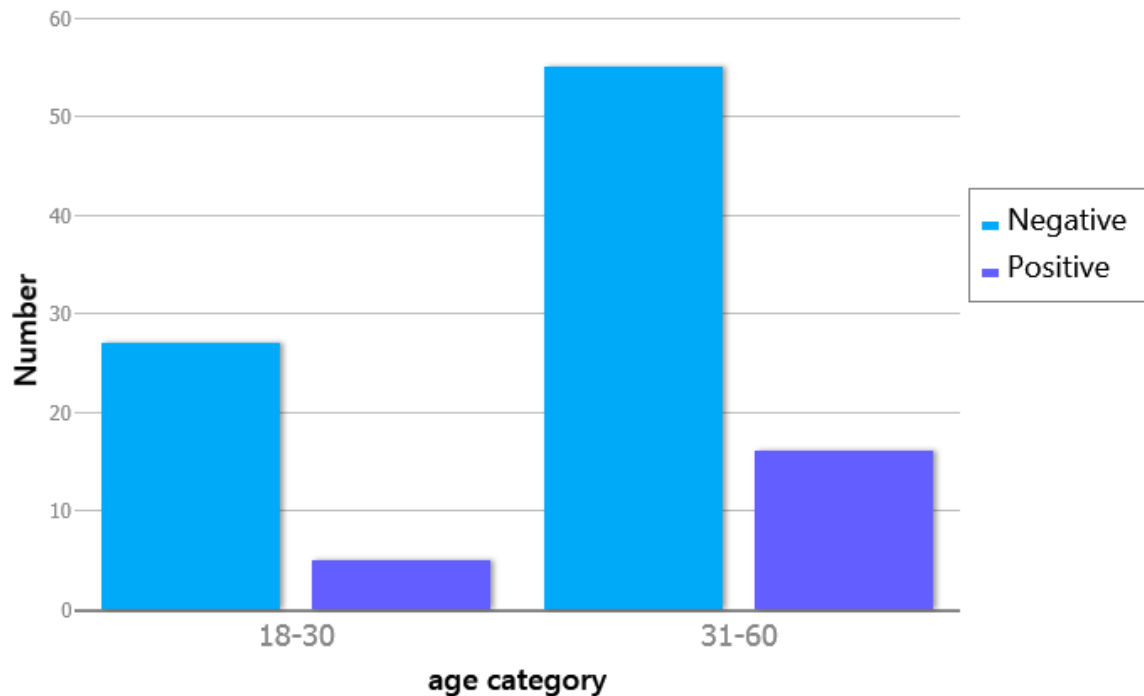


Figure 4-9: Distribution of seropositivity by age category.

The seroprevalence rate in the age group, 18-30 year and 31-60 year during the study period was 15.6% (5/32) and 22.5% (16/71). The difference was not statistically significant ($P=0.21$) and the odds of 18-30-year-old becoming infected with *Brucella* spp. are 0.64 times the odds of 31-60 year old becoming infected (Table 4.7).

The frequency and distribution of seropositivity of the 21 positive workers, according to the marital status for married workers was 8 (38.1%, 95%CI=18.1-61.6) and for single workers was 13 (61.9%, 95%CI=38.4-81.9) (Figure 4.10). The seroprevalence among the married workers and single workers during the study period was 19.1% (8/42) and 21.3% (13/61) respectively, this was statistically insignificant ($P=0.67$) and the odds of married workers becoming infected are 0.82 times the odds of single workers becoming infected (Table 4.7).

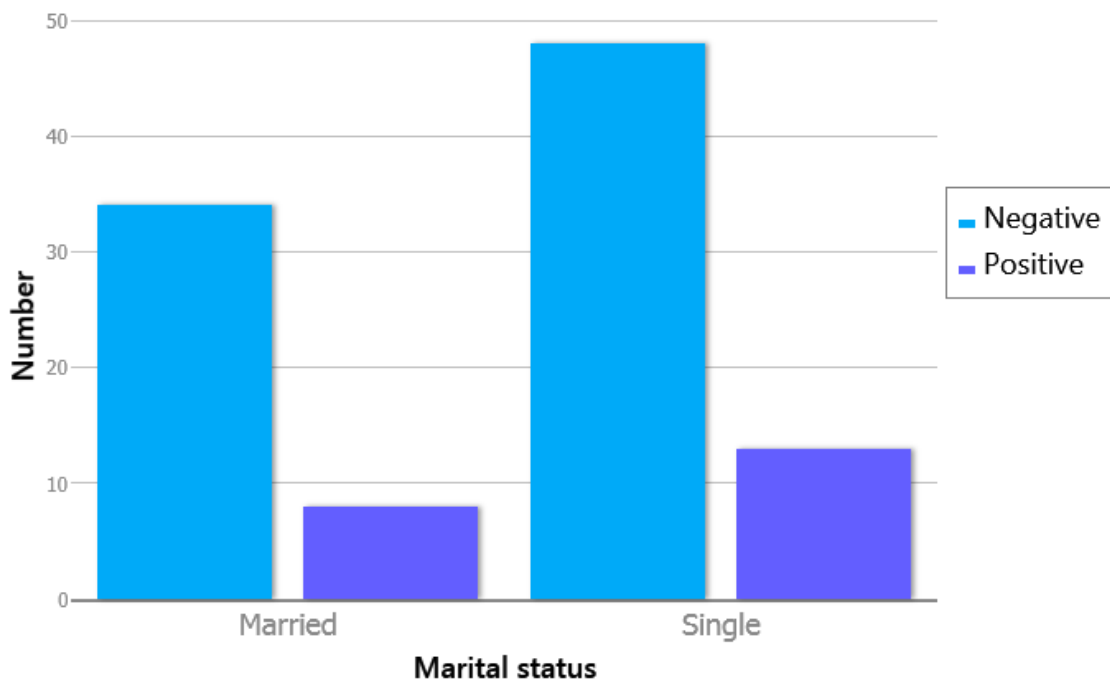


Figure 4-10: Distribution of seropositivity for brucellosis by marital status among abattoir workers.

Table 4.7: Bivariate analysis of demographic risk factors associated with seroprevalence of brucellosis among abattoir workers in Gauteng province, South Africa.

Variable	No. positive (%)	OR (95%CI)	Chi (X) ²	P-value
Sex				
Female	1 (6.25)	0.21 (0.08-0.55)	10.3	0.00
Male	20 (22.9)			
Age				
18-30yrs	5 (15.6)	0.64 (0.31-1.29)	1.15	0.21
31-60yrs	16 (22.5)			
Marital status				
Married	8 (19.1)	0.82 (0.42-1.61)	0.16	0.67
Single	13 (21.3)			

Of the 21 seropositive workers, the frequency and distribution of seropositivity of respondents stratified by the type of duties performed was as follows; Slaughter man (butchers) 15 (71.0%, 95%CI=47.8-88.7), Inspectors 2 (10.0%, 95%CI=1.17-30.4), Washing offal 2 (10.0%, 95%CI=1.17-30.4), Cleaners 2 (10.0%, 95%CI=1.17-30.4), Transporters 0 (0.0%, 95%CI=0.0-16.1), Managers 0 (0.0%, 95%CI=0.0-16.1) and Hides and skin 0 (0.0%, 95%CI=0.0-16.1) (Figure 4.11) .

The seroprevalence rate of the abattoir workers stratified by the type of duties performed was; Slaughter man (butchers) 22.1%, (15/68), Inspectors 14.3% (2/14), Washing offal 28.6% (2/7), Cleaners 25.0%, (2/8), Transporters 0.0% (0/2) Managers 0.0% (0/3) and Hides and skin 0.0% (0/1).

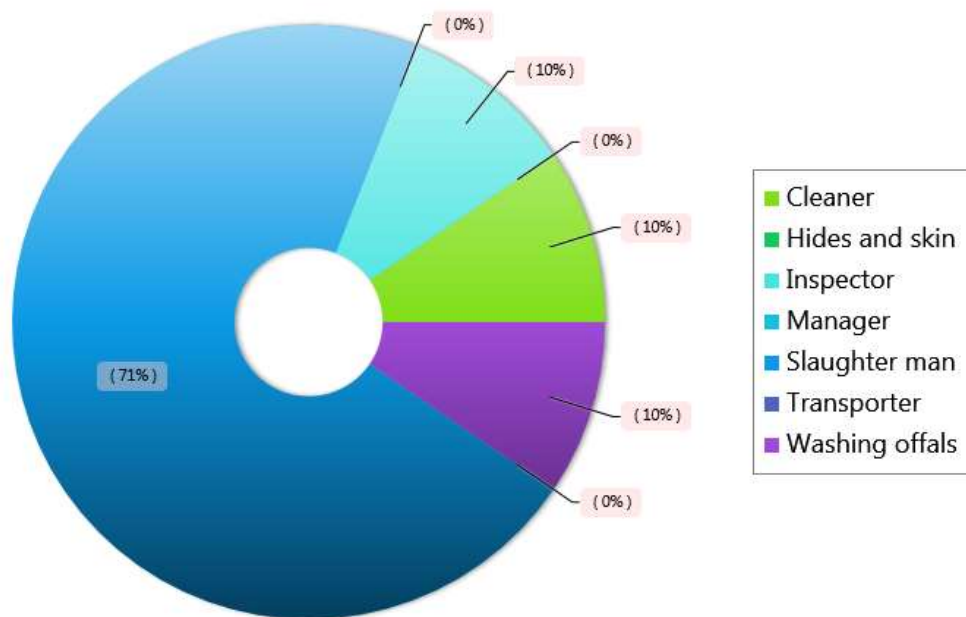


Figure 4-11: Distribution of seropositivity for brucellosis stratified by the type of duty description of the abattoir workers.

Of the 21 seropositive workers, and within the age categories stratified by the marital status of the respondents (Figure 4.12), the frequency and distribution of brucellosis was as follows; 18-30 years and married 1 (12.5%, 95%CI=0.32-52.7) and 18-30 years and single 4 (30.8%, 95%CI=9.09-61.4), while for 31- 60 years and married 7 (87.5%, 95%CI=47.4-99.7) and 31- 60 years and single 9 (69.2%, 95%CI=38.8-90.9). The seroprevalence rate of brucellosis among age categories stratified by marital status during the period of study for 18-30 year-old and married and 18-30-year-old and single was 12.5% (1/8) and 16.7% (2/24) respectively (Table 4.8), this was statistically insignificant ($P=0.55$). The seroprevalence rate of brucellosis among age category stratified by marital status during the period of study for categories 31- 60-year-old and married and 31-60-year-old and single was 20.6% (7/34) and 24.3% (9/37), respectively (Table 4.8), this was statistically insignificant ($P=0.73$).

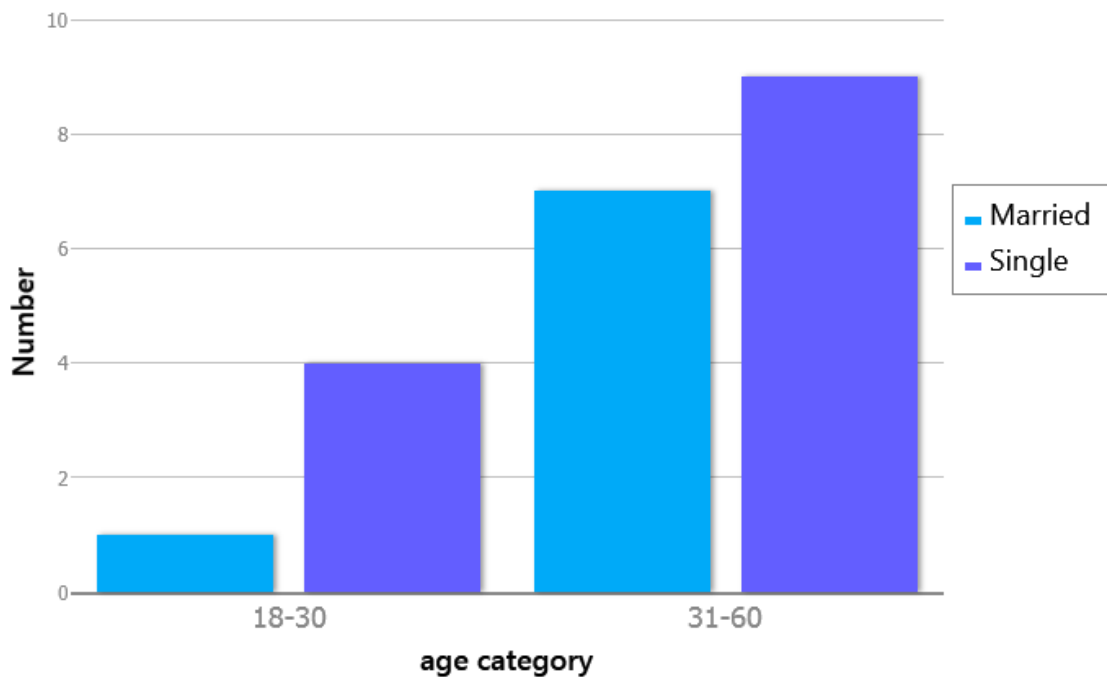


Figure 4-12: Frequency of distribution of brucellosis seropositivity among the abattoir worker within age category stratified by their marital status.

Table 4.8: Bivariate analysis of the frequency and seroprevalence of brucellosis among the abattoir workers according to age categories stratified by marital status.

Age category/ Marital status	No. positive (%)	OR (95%CI)	Chi (X) ²	P-value
18-30yrs. Married	1 (12.5)	0.73 (0.33-1.59)	0.35	0.55
Single	4 (16.7)			
31-60yrs. Married	7 (20.6)	0.84 (0.43-1.64)	0.11	0.73
Single	9 (24.3)			

Of the 21 seropositive workers, the frequency and distribution of brucellosis seropositivity among abattoir workers who worked for 5 to 7 days per week was 19 (90.0%, 95%CI=69.6-98.8) and those that worked for 1 to 2 days per week was 2 (10.0%, 95%, CI=1.17-30.4) (Figure 4.13). The seroprevalence rate of brucellosis stratified by the number of days at work per week by the abattoir workers was as follows among those who worked for 5 to 7 days 19.4% (19/98), for those who worked for 3 to 4 days 0.0% (0/2) and for those who worked for 1 to 2 days, 66.7% (2/3).

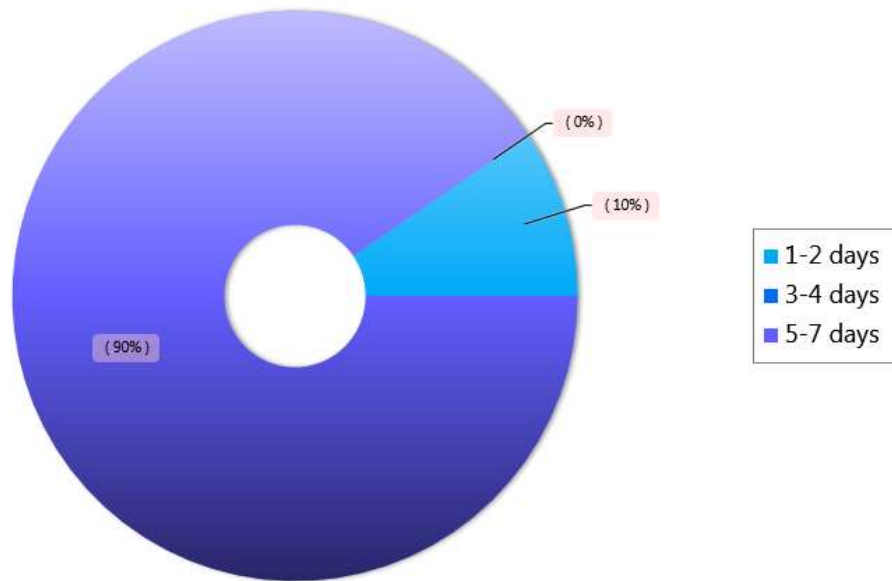


Figure 4-13: Seropositivity of brucellosis among abattoir workers by the number of days at work.

Of the 21 seropositive workers, the frequency of brucellosis seropositivity among the abattoir workers that have worked for 1 year at the abattoirs was 5 (25.0%, 95%CI=8.22-47.2), those who have worked for 2 year was 3 (14.0%, 95%CI=3.05-36.3) and those who have worked for 3 years and above was 13 (62.0%, 38.4-81.9) (Figure 4.14). The seroprevalence rate of brucellosis stratified by the number of years of working at the facilities among abattoir workers that have worked for 1 year was 23.8% (5/21), those who have worked for 2 years was 17.6% (3/17) and for those who have for worked for 3 years and above was 20.0% (13/65).

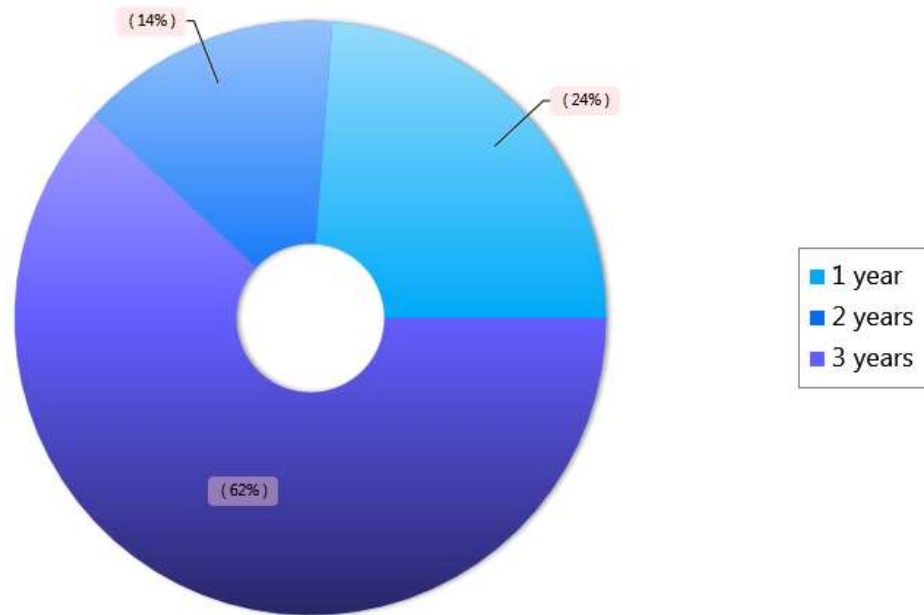


Figure 4-14: Seropositivity of brucellosis by the number of years at work.

4.4.7 Bivariate analysis of risk factors among the respondents associated with seropositivity

According to Table 4.9 illustrating the risk factors associated with seropositivity for brucellosis, 36.9% of respondents reported to be working at farms other than the abattoirs, there was an association with working on other farms with seropositivity ($P=0.04$, OR: 0.45, 95%CI:0.21-0.94). Respondents who consumed unpasteurized milk had a seropositivity of 25.2%, and there was no association between unpasteurized milk consumption and seropositivity ($P=0.85$, OR: 0.88, 95%CI: 0.44-1.76). Respondents who slaughtered animals at home were 11.7% and there was an association between slaughtering animals at home with seropositivity ($P=0.01$, OR: 0.31, 95%CI: 0.13-0.73). The respondents who consumed raw or undercooked meat made up 47.6%, and there was a marginal association of raw or undercooked meat consumption with seropositivity ($P=0.05$, OR: 2.10, 95%CI: 1.04-4.24). Of the respondents, 94.2% agreed to using PPE, and there was a negative association of PPE usage with seropositivity of brucellosis ($P=0.00$). Among the respondents, 26.2% have knowledge on brucellosis, and the odds of those who have knowledge of brucellosis becoming infected with brucellosis are 0.39 times the odds of those who have no knowledge of brucellosis becoming infected, there was an association between lack of knowledge and seropositivity ($P=0.02$, OR: 0.39, 95%CI: 0.18-0.85). The respondents who believed they could contract brucellosis from animals were 21.4%, and the odds of those who believed they could become infected from

animals becoming seropositive are 0.20 times the odds of seropositivity among those who did not believe they could become infected from animals, there was an association with negative perception of infection from animals with seropositivity ($P=0.00$, OR: 0.20, 95%CI: 0.09-0.44). Of the respondents, 74.7% experienced hand cut injuries at least once while performing their duties, and there was no association between hand cut injuries and seropositivity ($P=0.73$, OR: 0.84, 95%CI: 0.43-1.65). Of the respondents, 2.0% were previously diagnosed with brucellosis, and there was an association between previous brucellosis diagnosis with seropositivity ($P=0.00$, OR: 4.00, 95%CI: 2.14-7.49). Of the respondents, 77.7% experienced the splash of animal blood or fluid on their faces at least once while performing their duties, there was no association between splash of animals' blood on faces with seropositivity ($P=0.14$, OR: 0.53, 95%CI: 0.25-1.12) (Table 4.9).

Table 4.9: Bivariate analyses of risk factors associated with sero-prevalence of brucellosis among abattoir workers in Gauteng province, South Africa.

Risk factor	No. tested (% Seropositive)	OR (95%CI)	P-value
Working on farms besides abattoir? Yes No	38 (13.2) 65 (24.6)	0.45 (0.21-0.94)	0.04
Do you consume unpasteurized milk? Yes No	26 (19.2) 77 (20.8)	0.88 (0.44-1.76)	0.85
Do you slaughter animals at home? Yes No	12 (8.33) 91 (21.9)	0.31 (0.13-0.73)	0.01
Do you eat raw or undercooked meat? Yes No	49 (26.5) 54 (14.8)	2.10 (1.04-4.24)	0.05
Do you always wear your PPE? Yes No	97 (21.6) 6 (0.0)	undefined	0.00
Do you know of brucellosis? Yes No	27 (11.1) 76 (23.7)	0.39 (0.18-0.85)	0.02
Do you think you can get brucellosis from animals? Yes No	22 (9.1) 6 (33.3)	0.20 (0.09-0.44)	0.00
Have you had hand cut injuries on duty? Yes No	77 (19.5) 26 (23.1)	0.84 (0.43-1.65)	0.73
Have you ever been diagnosed of brucellosis? Yes No	2 (50.0) 101 (19.8)	4.00 (2.14-7.49)	0.00
Have you ever had animal blood splashed on your face? Yes No	80 (77.5) 23 (86.9)	0.53 (0.25-1.12)	0.14

4.4.8 Multiple logistic regression analysis of risk factors among the respondents associated with seropositivity for brucellosis

The univariable analysis shown in Table 4.10 indicated a significant association with seropositivity among the abattoir workers. All variables within a statistically significant level of

$P \leq 0.3$ were included in the multivariable logistic regression model analysis as shown in Table 4.11. None of the variables was significantly associated with brucellosis infection among the abattoir workers in the interactive multivariable analysis. The Hosmer-Lemeshow goodness of fit Chi-square test was not significant ($X^2=0.45$; P value = 0.98), indicating that the model fit the data well and had a high predictive ability. The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values were 106.80 and 119.97 respectively.

Table 4.10: Univariate analysis of factors determined to be significantly associated with human brucellosis positivity among abattoir workers in Gauteng derived through a Logistic Regression Model.

Variable	P-value
Sex	0.02
Job description	0.03
Days at work	0.04

Table 4.11: Multiple logistic regression of factors determined to be significantly associated with human brucellosis among abattoir workers in Gauteng derived through a Logistic Regression Model.

Variable	Odds Ratio	S E	P-value	95% CI
Sex	4.55	5.06	0.17	0.5, 40.1
Days at work	0.27	0.26	0.19	0.03, 1.87
Working on farms away from the abattoir	0.44	0.25	0.15	0.14, 1.34
Job description	1.10	0.72	0.88	0.31, 3.97

* S.E-standard error, CI-confidence interval, AIC value = 106.80; BIC value = 119.97.

4.5 Discussion

Data from the first phase of this study have been used to assess the knowledge among abattoir managers and staff on zoonoses as occupational hazards at the abattoir facilities and to assess the correlation between how the abattoir workers performed their duties while working at the abattoirs and the standards the managers had instituted for the protection and the public health safety of the abattoir workers. The managers, many of them who owned of the facilities, played leadership role in establishing the culture of infection prevention practice in accordance with the Occupational Health and Safety Act No. 85 of 1993 of the Republic of South Africa (SA-Act, 1993). These regulations are primarily for hazardous biological agents aimed at protecting the abattoir workers from infectious agents. The managers were also aware and had complied with the internationally recognized concept of animal disease prevention in humans, which is in line with the National Occupation Research Agenda (NORA), which stipulates that “veterinary doctors and other animal care personnel are at substantial risk for various occupationally acquired injuries and illnesses, many of which parallel and even exceed those encountered by human healthcare”(Scheftel et al., 2010).

From our data, the management of all 14 abattoirs provided PPE for their staff, they also expressed some knowledge about zoonoses and they indicated that they had passed on the knowledge on zoonoses to their workers. However, among the managers, 28.6% (4/14) agreed that there was a knowledge gap for Q-fever. The managers ascertained that none of the workers had been diagnosed of any zoonoses prior to our visit and they acknowledged some workers did engage in risky activities that could have predisposed them to infections while working at the abattoirs. These risky behaviours increase the exposure potential of these workers to occupationally acquired zoonoses. These occupationally related zoonoses have been reported in many studies around the world, such as Brazil, Nigeria, Iran, Pakistan and many more (Aworh et al., 2013, Esmaeili et al., 2016, Mukhtar and Kokab, 2008, Ramos et al., 2008).

These data resulted in the educational intervention aimed at bringing about awareness to both the managers and abattoir workers, and the primary goal was to enable them to be knowledgeable and be aware of the risks of zoonoses and injuries at the abattoir facilities.

Risk factors that could predispose these workers to infection were assessed, and this was because the transmission of diseases requires a source of infection (animals), a susceptible host (abattoir employee), a route of transmission (contact [direct or indirect], aerosol or vector-borne), and a portal of entry, such as an open wound or mucous membrane (Scheffel et al., 2010). In this study, the proportion of females was 22.0% among the respondents; this was significant because in some countries around the world, women are not allowed to work at the abattoirs due to some religious and cultural reasons, however, some religions have advocated for women to participate in the slaughtering of animals (Bonne and Verbeke, 2008). The proportion of 22.0% of female workers in our study was comparable to 20.0% proportion of female abattoir workers in another study conducted in South Africa (Nyamakwere et al., 2017). South Africa is championing gender equality and advocacy for women's rights (Kehler, 2001), and this is in accordance with the 17 sustainable development goals. Among our respondents, 37.1% were married (Figure 4.4), this is also significant to know because zoonoses such as brucellosis are now thought to be transmissible through coitus and if they test positive it would be easy to trace their partners for screening (Meltzer et al., 2010). This is also significant in non-married workers, because infected workers may have equal potential to transmit zoonoses like brucellosis to their partners as sexually transmitted infection. A large proportion (87.0%) of the respondents, were in the job category of slaughter man (Table 4.1) which is also known as the butcher in our context. This group was mostly in contact with the animals and could be more at risk of exposure to zoonoses via risky practices compared to the other groups within the job description context. In a study in Pakistan, a seroprevalence rate of 27.1% was reported in slaughterers in the abattoir workers underscoring the type of job

performed at the facility was significant in the occurrence of the disease in the workers (Mukhtar and Kokab, 2008). Ramos et al. (2008) in a similar study reported a 7.7% seroprevalence of brucellosis among those who slaughter and bleed animals in abattoirs in Brazil. This was fairly higher than what was observed in other job description in the study.

In our study, 84.0% of our respondents confirmed to have had hand cut injuries at least once while performing their duties at the abattoirs. These cut injuries are portals of entry for infectious organisms when in direct or indirect contact with infected animal's blood or fluid. This study stratified the cut injuries among our respondents according to gender, of which cut injuries were higher in the male workers at 75.0% (Table 4.2), although in our study, the males were more than the females which could be a factor. A plausible reason may also be that the females are thought to be more cautious than their male counterparts, and among the age categories, cut injuries were higher at 100% within the age category 61 years and above, this can be attributed to higher exposure potential by increased years of work. Cut injuries in relation to the duration of work years within those who have worked for more than one year or less than three years was highest at 88.2%, this may be explained as a co-founding variable to hand cuts. In relation to the type of abattoirs, respondents in high throughput abattoirs experienced more hand cut injuries at 55.8% compared to those in the LT abattoirs. This may be as results of a greater number of animals being processed at high throughput facilities compared to the LT abattoirs. Another reason may be that carcass conveyor in the high-throughput abattoir is likely to be moving at a relatively faster rate compared to LT; implying operations are conducted with relatively high speed compared to LT. Further, long working hours are likely to be associated with HT abattoirs making operatives to work under fatigue which may increase the risk of cut injury.

A proportion of 91.0% among our respondents confirmed to have experienced splash of animal blood or fluid on their faces while performing their duties at the facilities. The splash of animal secretion is significant because, the infectious organisms can directly come in contact with the mucous membrane of the eye conjunctiva or any open wound leading to infection (Corbel, 2006). Further, splashed blood or fluid may stain and be retained on the PPE of workers and if precaution is not taken could serve as a source of infection (Corbel, 2006); This is particularly important considering that 38.5% of our respondents agreed to eating during the break without removing their PPE which was a risky practice. Most of our respondents indicated that they used PPE, which in our context referred to the usage of aprons, hair nets, beard nets and overall gowns; however, only 0.70% stated that they did not use PPE regularly. The 0.70% non-compliance of PPE usage is lower than the 11.3% reported for abattoir workers in Ethiopia (Mekonnen Haileselassie et al., 2013) and considerably lower than the 62.2% reported for abattoir workers in Nigeria (Adesokan et al., 2018). The implication of non-

compliance is an increase in zoonoses exposure potential, and an increase in the contamination of carcasses with microorganisms from meat handlers, especially from those who do not use hand gloves.

A proportion of 70.0%, 58.0% and 90.2% of our respondents did not use facial masks, hand gloves and goggles respectively (Table 4.1). Esmaeili et al. (2016) had reported that Iranian abattoir workers were exposed to infectious agents through splash on their faces of which the eye may have served as a portal of infection. This was possible because their faces were not protected by goggles.

Of the respondents, 67.1% had experienced symptoms of acute febrile illness (fever, flu, headaches, night sweat amongst others). It was observed in the current study that a large proportion of 88.1% of the respondents did not seek medical help whenever they felt sick, and this underscores the gap in the knowledge of seeking professional help among these workers. This type of behaviour has been reported in a similar study in South Africa, of which a 74.1% of abattoir respondents did not feel the need for medical health intervention when they fell ill (Nel et al., 2004). Similarly, studies in Nigeria and Ethiopia had also reported that 50.6% and 7.7% of respondents respectively did not seek medical intervention when they experienced symptoms of illnesses (Adesokan et al., 2018, Mekonnen Haileselassie et al., 2013). This is significant because zoonoses such as brucellosis express as an undulating fever, and probably when the workers felt some relief from the fever, they thought the disease episode was over, and this causes relapse and complications due to chronic infection (Corbel, 2006).

In our study, 1.4% of the respondents confirmed stealing condemned meat from the abattoirs, while 6.3% of the respondents also indicated that they consumed raw meat at the abattoirs, a practice that considerably increases their exposure potential to many foodborne zoonotic pathogens. Aworh et al. (2013) reported the association of brucellosis to the consumption of raw meat in West Africa and it is therefore essential to make abattoir workers aware of the risk of this practice as highlighted in this study.

In the current study, 37.1% of the respondents had the perception or belief that they cannot get sick from animal diseases while working at the abattoirs, a finding similar to the report that 31.3% of abattoir workers in China who believed that infection from animals had nothing to do with their daily lives and work (Hao et al., 2008). The perception of an issue determines how an individual would respond or react to the issue, as such, if the respondents did not perceive they can get sick while working at the abattoirs, they would probably not take preventive measures seriously at the facilities.

All the risk factors measured in the current study were comparatively higher among respondents from HT abattoirs (88.0%). The high rate of defaulters in the HT abattoirs in our study was in contrast to what was reported in another abattoir study in South Africa, of which an equal proportion of defaulters were observed in both HT and LT abattoirs (Nyamakwere et al., 2017). The proportion of those who did not report illnesses in the study by Nyamakwere et al. (2017) in the HT abattoirs was 24.0%, and this was lower than 60.3% reported in our study. In the LT abattoir in the same study by Nyamakwere et al. (2017), a proportion of 5.0% did not report illnesses and was comparatively lower than 39.7% of abattoir workers in our study who did not seek or report illnesses when they felt ill.

Respondents (100.0%) who did not use face goggles were among those who had worked for two years; and non-usage of goggles was higher at 100.0% within the age category 61 years and above. None compliance with goggle usage is a highly likely risk factor for infection through the conjunctiva of the eye. Infection rate of brucellosis have been reported to be higher in older workers than younger ones as reported by Ramos et al. (2008) showing infection rate of brucellosis to be 2.9%, 3.4% and 8.0% among age groups of 18 to 30 year-old, 31 to 40 year-old and 40 years and above, respectively. This is probably because the older the worker the more the chances they have worked more years at the abattoir, hence more exposure potential.

The proportion of respondents who experienced symptoms of febrile illnesses was at 39.0% higher within those who had worked for three years and above, and at 35.0% within the age category 31-60 years. Experience of acute febrile symptoms is significant because these may be symptoms emanating from zoonoses infection. Brucellosis was reported among 26.2% of abattoir workers in Brazil who agreed to have experienced febrile symptoms (Ramos et al., 2008). This was closely related to the number of those who experienced such symptoms in our study. Brucellosis in human may be subclinical and in many cases, humans test positive serologically without apparent clinical symptoms (Marques et al., 2000). This type of unnoticed infection is very common in abattoir workers and other professionals who work with animals (Bennett and Plum, 1997, Keane, 1982).

It was observed that those who had worked for only one year or less were more at risk of infections in relation to their attitude and practices; this may be because they were naïve and new to the working environment, as such they had no experiences of occupationally related safety issues yet. Ramos et al. (2008) reported that 78.4% of abattoir workers did not know how they could get infected with brucellosis while working at the Brazilian abattoir, and 4.6% of these workers tested positive and this was higher compared to 4.0% positivity among those who knew how infection could be spread from animals to humans at the abattoirs.

The current study among all respondents based on their years of work at the abattoirs, revealed a trend of higher association of risk practices among those who had worked for three years and above. The workers who had worked for more than three years and above were more likely to have experienced all the risk factors measured in our study. This can be attributable to the fact that they may have experienced at least once either of the inappropriate practices at the abattoir or they may have taken for granted the fact that they have become comfortable with working for this long duration and not paying much attention to all the precautionary measures that had been put into place. Another reason may be that a person who has worked long in an abattoir had probably handled the knife more times than a recent employer. Therefore, the risk of exposure may not be necessarily linked to the attitude of the worker but to the frequency of handling the knife. If the recent worker stays long in his job, he is likely to cut himself because of the increased contact with the knife. Ramos et al. (2008) in the abattoir study in Brazil reported an increase in the prevalence of brucellosis in abattoir workers from 4.9% in those who had worked for 1 to 10 years to 5.3% in those who had worked for 10 years and above. Nyamakwere et al. (2017) reported that those who had worked for five years and above had experienced more training sessions to prevent infections compared to those who had worked for less than five years at the abattoirs in their study.

This study produced evidence-based data for the sensitization of the abattoir workers. It revealed that most of the workers had not taken seriously the preventive measures to mitigate injuries or zoonotic infections while working with animals, sharp instruments or equipment at these facilities. Hence, the sensitization of the managers and abattoir staff was justified.

This phenomenon of enlightenment through the campaign was in line with the vision of the veterinary public health which started in 1948 with the World Health Organization (WHO, 1959). The veterinary public health has developed and metamorphosed into inculcating the concept of One Health to improve the wellbeing of animals, humans and the environment; and to bring about awareness and recognition of zoonoses and animal related injuries as occupational risks and this has called for the need for specific knowledge to ensure the prevention and control of such zoonotic infections and injuries (Battelli, 2008). The sensitization campaign fulfilled this concept and the knowledge retention could be evaluated in the second phase.

In the second phase, most of the respondent had forgotten about the zoonoses awareness lessons. This indicated that the knowledge retention was poor and repeated training and re-training would improve the retention of knowledge gained through training.

The knowledge gap observed in the current study may be attributed to the long interval of 12 months between both phases of the study (i.e. first phase of sensitization and the second

phase of knowledge). This may be one of the limitations of our study, and this probably reflected the lack of commitment by the managers on the training and re-training of their staff after episodes of training. The possibility of forgetting what was taught could have been reduced if training was conducted between the first sensitization training and the subsequent knowledge retention interview. One of the perceived problems is that the education brochures were made of paper material, which could have been decimated by water or fluid at the abattoirs or at the homes of the respondents, however, if the materials were made in form of a glossy booklets, the material would have lasted longer and chances of refreshing their memories would have been higher. Furthermore, the posters distributed were only placed in the manager's offices, with the workers having limited access to the information on the posters. The display of the posters at locations such as the tea/coffee/lunch rooms readily accessible by the workers would have facilitated the workers to read and refresh their memories. It was also observed in the current study that some of the respondents that were taught in the first phase had left the abattoirs for other jobs, as such, the number of those sensitized earlier was reduced, and the new staffers were probably not informed about our previous campaign. This apparent loss of trained workers replaced by new untrained workers underscores the fact that the managers or abattoir owners need to be more engaged in prompt education of new staff to breach the knowledge gap on zoonoses and consequently reduce the risk of zoonoses posed to the new workers. The memory retention interview conducted in this study has shown that there is a need to adopt a better method to convey knowledge to abattoir workers to enable them to retain the information conveyed to them. An improved approach may assist in the retention of knowledge acquired during training sessions; this may involve the training of workers on a frequent and regular basis and the use of both print and electronic media.

Data from the second phase of this study investigated the brucellosis seroprevalence of abattoir workers in Gauteng province of South Africa and the risk factors associated with seropositive individuals. The seroprevalence rate of brucellosis using the overlap of either of the serological tests (RBT, BrucellaCap, IgG ELISA) among abattoir workers in the province was found to be in a total of 20.4%. The risk factors found to be associated with seropositivity were sex, job description, days at work and working on other farms apart from the abattoir. This is the first abattoir study on human brucellosis to be conducted among abattoir workers in South Africa, thereby providing relevant data on this group of workers and underscores the urgent need of replicating the study in other provinces, to fully understand the status of the disease among the occupationally exposed group in the country. This becomes highly imperative as the disease is known to be endemic in the country. This has observed the exposure of the workers to infection either at the abattoir or away from the facility.

The seroprevalence rates of 17.5% (ELISA) and 12.6% (RBT) in this study was comparable to a range of seroprevalences reports from other studies around the world; of which seroprevalence rates of 21.7% (ELISA), 25.5% (ELISA) and 19.5% (RBT) were observed among abattoir workers in Pakistan, India and Tanzania respectively (Barbuddhe et al., 2000, Mukhtar and Kokab, 2008, Swai and Schoonman, 2009). Although these are comparatively higher than the seroprevalence results from ELISA and RBT tests used in this study, some other studies have, in contrast, reported lower seroprevalences of 7.9% (ELISA) and 9.4% (RBT) among abattoir workers in Iran and Egypt respectively (Abdelbaset et al., 2018, Esmaeili et al., 2016).

The participation of both genders in the abattoir activities as observed in our study is of significance. Out of the 103 workers tested, females and males accounted for 15.5% and 85.5% respectively. A higher seroprevalence was recorded in males (22.9%) compared to females (6.25%) workers. The 95.2% proportion of seropositive males in our study is comparable to the 87.0% reported for male workers in abattoirs in Nigeria (Aworh et al., 2013). Furthermore, the overall seroprevalence of brucellosis in our study is significantly higher ($P=0.00$) in male (22.9%) than in female (6.25%) workers coupled with the fact that the odds of females becoming infected with brucellosis are 0.21 times the odds of males becoming infected. This maybe explained as anecdotal belief suggests that females are more careful than males. Our findings are partly in agreement with the report of a study in Brazil where Ramos et al. (2008) reported low seroprevalence of brucellosis in female (2.0%) and male (4.0%) abattoir workers. The participation of female members of the population in the economy of South Africa has been on the increase which has contributed to the empowerment and improvement of the female gender's means of livelihood and enables them to contribute to the economy of the country as a whole (Kehler, 2001). This study investigated the association between the age of abattoir workers and infection with brucellosis, and it was detected that 15.6% of the workers in the 18-30 years age bracket were seropositive, a finding close to the reported seroprevalence of 16.1% detected in abattoir workers between the ages of 21-30 years in Pakistan, however, a lower seroprevalence of 2.9% was reported among workers in the 18-30 year age group in a study in Brazil (Mukhtar and Kokab, 2008, Ramos et al., 2008). This may in part suggest that the younger age group in Brazil may have reduced their exposure potential compared to our study and that in Pakistan.

The seroprevalence rate among the job description group of butchers in our study was 22.1% (RBT/ELISA), this was comparatively lower than 64.8% (RBT/ELISA) seroprevalence rate reported in 'slaughter men' in the Nigerian abattoir study (Aworh et al., 2013). However, the 22.1% seroprevalence rate of brucellosis among the butchers in our study was much higher than the 9.3% and 7.7% seroprevalence rates in slaughterers observed in the Iranian and

Brazilian abattoir studies (Esmaeili et al., 2016, Ramos et al., 2008). In these studies, and this abattoir study, most of the workers whose primary job was stated as slaughter men or slaughterers or butchers, experienced high infection rate compared to other job groups. This may be because of frequent handling of knives by slaughter-men compared to other job description.

Inspectors and those who wash offal in our study had seroprevalence of 14.3% and 28.6% respectively which are higher than what was observed among the inspectors and gut cleaners or offal washers in Brazil where seroprevalence rates of 1.5% and 1.3% respectively were observed (Ramos et al., 2008). These reports emphasize the fact that workers performing these kinds of duties at the abattoir facilities are also at risk and are also susceptible to occupationally acquired infections at the facilities.

Among the workers surveyed in this study, individuals who had worked for over 3 years were more (63.1%) and most importantly, the seroprevalence of brucellosis was highest (61.9%) in this group of workers. Our findings agree with the report of a study on abattoir workers in Brazil where higher seroprevalence of brucellosis was detected in those who have worked for longer years (Ramos et al., 2008). This study finding may be explained in part by the possibility that individuals who have worked for long periods at the abattoirs are most likely to become infected if exposed to infected livestock slaughtered at the abattoirs, resulting in increased exposure potential and experience compared to those who have worked for fewer years. Seroprevalence rate among those who worked for 1-2 days in a week was 66.7% and this was higher than 19.4% seroprevalence rate observed in those who worked for 5-7 days a week at the abattoirs, which was found to be significantly associated with seropositivity ($P=0.04$) in the univariate analysis (Table 4.10). It is plausible that these workers who work for fewer days may engage in working at other places which could predispose them to brucellosis apart from the abattoirs. Most of the abattoir studies on human brucellosis have not considered days at work as a risk factor to brucellosis infection and the results could be influenced by other factors not considered such as other activities leading to exposure away from the abattoir those that are working for only 1-2 days.

The bivariate analysis of the risk factors to brucellosis among the abattoir workers indicated seroprevalences among those who worked on farms not affiliated to the abattoirs and those who did not work on other farms were 13.2% and 24.6% respectively, this was statistically significant ($P=0.04$). The odds of those who worked on farms having brucellosis is 0.45 times the odds of those who did not work on farms having brucellosis. This may be suggestive of infection being acquired outside the abattoirs, and plausible by contact with infected animals on the farms rather than at the abattoirs.

Drinking unpasteurized milk has always been implicated since the discovery of brucellosis in humans (Corbel, 2006, Zammit, 1905). Among the abattoir workers in Brazil, a seroprevalence rate of 3.7% was reported among those that consumed unpasteurized milk which is lower than the 19.2% observed in this study. However, the observed 19.2% seroprevalence rate is comparatively lower than the 72.2% seroprevalence rate reported in those that consumed unpasteurized milk in the Nigerian study (Aworh et al., 2013, Ramos et al., 2008). A cultural practice of drinking unpasteurized milk is common in Nigeria, especially among the nomads (Bertu et al., 2010). This low seroprevalence rate among those who consumed unpasteurized milk in South Africa may be due to the fact that South Africa had always promoted the drinking of pasteurized milk since the scorch of brucellosis in the past (Strachan, 1932). However, high a risk of exposure is probable for those who do not adhere to drinking pasteurized milk in the country.

The seroprevalence rate among workers that consumed raw or undercooked meat and workers that did not was 26.5% and 14.4% respectively, which was statistically significant ($P=0.05$). The seroprevalence of 26.5% in our study is closely related to what was observed among abattoir workers that ate raw meat with a seroprevalence rate of 22.2% in the study in Nigeria (Aworh et al., 2013). Seropositivity rate for brucellosis has been reported to be higher in those who ate raw meat compared to those who did not in this study and the one conducted in Nigeria. Seroprevalence among individuals that slaughtered animals both at home and at the abattoirs was 8.33%, and the odds of individuals that slaughtered animals at home becoming infected with brucellosis was 0.31 the odds of those individuals that only slaughtered animals at the abattoirs becoming infected with brucellosis, this was statistically significant ($P=0.01$). Appropriate facilities and protective clothing most probably were not used for home slaughtering, and this may predispose to infection at homes. This may also suggest that those who slaughtered animals both at home and at the abattoir had increased chance of becoming infected with brucellosis than those who slaughtered only at the abattoirs.

Amongst the abattoir workers sampled, 94.2% indicated the constant use of PPE. It was however, unexpected to have detected that the seroprevalence of brucellosis in PPE users (21.6%) was significantly higher ($P=0.00$) than found in non-compliant PPE users (0.0%). The findings in our study are comparable to what was observed in the study in Iran on the use of PPE where the authors reported a seroprevalence of 13.7% and 2.2% in PPE and non-PPE users respectively (Esmaeili et al., 2016). This may suggest that workers, who used PPE and were seropositive, may have been infected via another means; for example, 24.8% of respondent reported in our study, who always wore PPE, were engaged in drinking unpasteurized milk, and were among respondents who tested positive for brucellosis in the study.

Among all the respondents in our study, 26.2% had knowledge of brucellosis, and the seroprevalence of brucellosis in workers with knowledge of brucellosis was significantly ($P=0.02$) lower (11.1%) than detected in workers without the knowledge of the disease (23.7%). In agreement with our study, Ramos et al. (2008) in an abattoir study in Brazil also reported a lower seroprevalence of brucellosis in workers who had knowledge of brucellosis (4.1%) than in workers who did not (6.5%). The knowledge of zoonosis is important as this will inform the workers on how to better protect themselves as they perform their duties at the abattoir facilities. A similar pattern was observed regarding workers' beliefs or perceptions on contracting brucellosis from animals. Among the workers surveyed, 21.4% believed that they could contract brucellosis from animals and the seroprevalence of brucellosis in this group of workers (9.1%) is significantly ($P=0.00$) lower than the 33.0% detected in workers who did not believe or perceive that they can contract brucellosis from animals. Our findings are at variance with the report of Ramos et al. (2008) in which similar seroprevalence was detected in abattoir workers who knew how brucellosis spreads (4.0%) and in those who did not (4.6%) in a study conducted in Brazil. It is well known that people's knowledge, attitude and perception (KAP) are important for successful prevention and control of diseases including zoonoses such as brucellosis (Noe et al., 2006). This is because people's perception determines how they will react to issues regarding the risks of working with animals at the abattoirs.

In this study, overall 74.7% of the abattoir workers reported having experienced hand cut injuries at least once while performing their duties and the seroprevalence of brucellosis in workers who had experienced hand cuts and those who have not, was 19.5% and 23.1%, respectively but the difference was not statistically significant ($P=0.73$). However, a study on abattoir workers in Nigeria reported very high seroprevalences of brucellosis in the two groups of workers, 63.0% and 44.1% in workers who experienced hand cut injuries and those who did not, respectively (Aworh et al., 2013). It has been documented that *Brucella* spp. can infect humans through compromised skin, especially when a worker has a cut injury and continues working at the abattoirs. Compromised skin in contact with infected animal's secretions is known to be one of the ways brucellosis takes place in the human (Corbel, 2006).

It was not a surprise that the seroprevalence of brucellosis in abattoir workers who had indicated previous diagnosis of the disease was 50.0% while in workers without a prior diagnosis of brucellosis, it was 19.8% and the difference was statistically significant with an OR of 4.00 ($P=0.00$). This is plausible because brucellosis is very difficult to treat, and the antibodies remain in the system for a very long period of time, even after treatment, serology will still show antibodies due to the previous exposure (Corbel, 2006).

The risk factors measured in our study were fitted in a multiple logistic regression model which found sex, job description and working on farms away from the abattoirs to be insignificantly associated with human brucellosis among the abattoir workers in the Gauteng province. Days at work were statistically associated with *Brucella* seropositivity and this relationship could be non-causal. Some of these risk factors have also been associated with the occurrence of brucellosis in other countries (Aworh et al., 2013, Esmaeili et al., 2016, Ramos et al., 2008). It cannot be over-emphasized that interventions need to be done to reduce the impacts of these identified risk factors as part of an overall objective to reduce the exposure potential and exposure experience of abattoir workers to brucellosis.

4.6 Conclusions

In conclusion from the first phase, this study revealed that most of the abattoir workers had defaulted in many preventive measures to infections at the abattoirs. It is plausible that many of the abattoir workers may have at one point potentially been exposed to zoonoses while working at abattoirs. It cannot be over-emphasized that some of these risk factors should be taken seriously, especially with the perception of workers that they cannot acquire animal diseases due to their activities at the abattoirs. With the increased knowledge now available globally, it is not acceptable for the knowledge gap detected in the current study to be left unaddressed. Routine and regular training and re-training of abattoir managers and their staff are imperative considering that a clear majority of the workers were unable to recall information conveyed to them on selected zoonoses during the sensitization campaign 12 months prior to the memory retention interview. It is suggested that in future studies, pre-KAP and post-KAP (knowledge, attitude and perception) assessments should be conducted to ascertain the knowledge gap before training and after training, as long time assessment may not reflect the actual success of training. Finally, it is pertinent to conduct provincial and nationwide surveys of these risk practices among abattoir workers and to conduct an abattoir based human zoonoses prevalence studies with test data from the managers to ascertain the prevalence of zoonotic diseases in abattoir workers in the country.

In conclusion from the second phase, this study has demonstrated, for the first time in South Africa, the occurrence of human brucellosis in abattoir workers. This has also achieved the objective of the study to determine the seroprevalence of the brucellosis among abattoir workers in Gauteng abattoirs where positive animals were detected from 2017 to 2018 in the previous objective. This suggests in part a source attribution to animals slaughtered at the abattoirs. However, some risk factors measured have shown that infection among the abattoir workers may have been due to other risk factors outside the abattoir facilities. The

multidisciplinary One Health approach has been successfully applied in this study and the interaction between animals and humans in the abattoir environment has been evaluated to be a plausible source of brucellosis to the workers. This underscores the fact that the abattoir facilities can be used for active and passive surveillance of diseases of public health and economic importance. This study has provided an early warning sign of potential transmission of zoonoses to humans who work in the abattoirs. The evidence-based data generated in our study has provided baseline data for policy makers to develop solutions to mitigate the further spread of brucellosis among abattoir workers, and to ensure proper management of the abattoirs, including the enforcement of preventive practices to reduce the infection rate among these workers. A structured One Health approach sensitization campaign as an intervention tool to reduce the risks posed to abattoir workers, from occupationally acquired zoonoses will help prevent infection among the population at risk. Regular reporting of acute febrile illness combined with serology (15-30 days of interval), isolation of *Brucella* and treatment as recommended by the WHO of abattoir workers should be encouraged and practiced as a monitoring tool for the detection of brucellosis and the associated risk factors in South Africa. This study has determined for the first time in South Africa, the occurrence of antibodies against brucellosis among abattoir workers, which could be because of infection or previous exposure to *Brucella*. This underscores the fact that the abattoir facilities can be used for active and passive surveillance of diseases of public health and economic importance. The evidence-based data provided by our study will be invaluable to policy makers. We recommend the implementation of brucellosis testing of abattoir workers country wide to establish base-line data that could indicate previous exposure, and that could be used to mitigate appropriate preventive practices to reduce the infection rate among these workers as well as provide insight into the magnitude of infections by *Brucella* spp. among the abattoir workers.

4.7 References

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Chapter 5

Seroprevalence and Characterization of *Brucella* species in cattle slaughtered at Gauteng abattoirs, South Africa

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5.1 Abstract

Brucellosis is an infectious and contagious zoonotic bacterial disease of both humans and a wide range of domestic and wild animals. In low and middle income countries (LMIC) where brucellosis is endemic, baseline data on the prevalence of the disease is lacking. Abattoir facilities can be used for active and passive surveillance to generate data on the occurrence of brucellosis in animals supplied to the abattoirs by farms and/or feedlots. A cross-sectional, simple random sampling method was used and un-clotted blood samples with corresponding tissue samples (lymph nodes, spleen and liver) were collected. Serological, molecular and bacteriological methods were used to detect *Brucella* spp. from 200 cattle. DNA detection and *Brucella* isolation were performed regardless of the serological status, and characterization of *Brucella* to genus, species and biovar (bv) level was conducted on isolates from 14 consenting abattoirs in the Gauteng province of South Africa between April 2016 and April 2018. Screening by Rose Bengal test (RBT) revealed a seroprevalence of 11.0% (22 of 200) with 95% CI of 6.69% to 15.75%, iELISA confirmed 5.5% (11 of 200) with 95% CI of 2.78% to 9.63% as estimated seroprevalence and CFT confirmed only 18.2% (4 of 22) of RBT-positives. ITS-PCR assay on tissue samples detected *Brucella* DNA in 12.5% (25 of 200) with 95% CI 8.26% to 17.9% and AMOS PCR characterized *Brucella* DNA of 11 isolates from cattle as *B. melitensis* (n=6) and *B. abortus* (n=5). Five (all from seropositive animals) isolates were bityped consisting of two *B. abortus* biovar (bv) 1 and three *B. melitensis* (n=3) isolates of which one was biovar 2 and two were biovar 3. The implication of these findings is that an abattoir study can provide invaluable data on disease surveillance. Furthermore, when *Brucella*-infected animals are slaughtered, they can serve as sources of infection to

susceptible abattoir workers and consumers of undercooked meat and unpasteurized milk or milk products from these animals.

5.2 Introduction

Brucellosis is an infectious and contagious zoonotic bacterial disease of humans and a wide range of domestic animal and wildlife, particularly the ruminants (Corbel, 2006, Radostits et al., 2006, Smirnova et al., 2013) and some marine animals (Foster et al., 2007, Scholz and Vergnaud, 2013). The *Brucella* species are Gram-negative, non-capsulated, facultative intracellular, non-spore forming, cocco-bacilli bacteria (Godfroid, 2012, Seleem et al., 2010, Smirnova et al., 2013). *Brucella* spp. infecting farm animals include *B. abortus*, *B. melitensis*, *B. suis* and *B. ovis* (Godfroid et al., 2010, Smirnova et al., 2013). The *Brucella* species are known to have host preferences, although there could be cross-infection with other hosts. *Brucella abortus* has a host preference for the cattle but it can cause infection in other hosts including humans (undulant fever). *Brucella melitensis*, has a host preference for sheep and goats and it is the most pathogenic of the *Brucella* spp. that causes infection in humans (Malta fever). *Brucella canis* and *B. suis* has host preferences for dogs and pigs respectively and can cause brucellosis in humans (Alton, 1990, Carmichael, 1990, Godfroid et al., 2005, Pappas, 2013). Some of the clinical signs seen in infected animals may present as an abortion storm (Bang's disease) especially in cattle. Infected cows may abort in first pregnancies, but in most cases, the fetuses are carried to full term in subsequent pregnancies (Corbel, 2006). Brucellosis has been eradicated or well controlled in developed countries (Pappas et al., 2006). However, in many of the LMIC such as in Africa, South and Central America, Middle East, Asia, Mediterranean Basin and the Caribbean, brucellosis is still common and high in occurrence both in the animal and human populations (Adesiyun and Cazabon, 1996, Godfroid et al., 2005, Olsen and Palmer, 2014, Pappas et al., 2006). In many of these LMICs where brucellosis is endemic, baseline data on the disease prevalence is lacking or unreliable. As such, an abattoir surveillance study on brucellosis can generate evidence-based and baseline data on the occurrence of the disease among the animal population, especially when the animals come from various farms to be processed into wholesome meat products for human consumption (Alton et al., 2015, Fasina et al., 2015, Kaneene et al., 2006). An estimation of over 3,476,000 of cattle were slaughtered as of August 2015 in the abattoirs in South Africa (DAFF, 2016). These abattoir facilities can also be used to monitor disease control policies, detect newly introduced disease agents, and to assess intervention programmes, such as brucellosis vaccination, and most importantly, abattoir survey may also facilitate early intervention to mitigate the epidemic loss of animals (Alton et al., 2015, Fasina et al., 2015, Kaneene et al., 2006). Carcasses of *Brucella*-infected slaughtered animals can

be a source of infection to susceptible abattoir workers; as these workers may be exposed to infection through direct contact with infected animal's secretions or blood, or indirectly through the consumption of raw meat or undercooked meat (Corbel, 2006).

In South Africa, brucellosis is a reportable and priority disease. Brucellosis may have existed in South Africa as an ancient disease as suggested by a paleopathological analysis study on the fossil of the late *Pliocene hominin* species (D'Anastasio et al., 2009). Humans have been detected to be infected with brucellosis in South Africa through the consumption of unpasteurized milk since the 18th and 19th centuries, and animals have been reported to be infected from an abattoir study many years ago in the country (Bishop, 1984, Strachan, 1932, Van Drimmelen, 1951). Many control measures have been instituted to prevent the spread of bovine brucellosis in the country. The focus is primarily on bovine brucellosis with little attention paid to brucellosis in other animals such as sheep, goats, pigs and wildlife. Currently, the principles, objectives and proposed direction for reviewing the approach to bovine brucellosis control in South Africa are being carried out to replace the old control methods for bovine brucellosis, including the regulatory framework. The goal of this initiative, being conducted by the Department of Agriculture, Forestry and Fisheries (DAFF), is to improve existing control measures for brucellosis in the country. In addition, DAFF (2017) is reviewing the following legislations, specifically: Animal Diseases Act (Act 35 of 1984), Animal Diseases Regulations (R.2026 of 1986), Bovine Brucellosis Scheme (R.2483 of 9 Dec 1988), Bovine Brucellosis Interim Manual.

In South Africa, testing scheme for bovine brucellosis (established under section 10 of the Animal Disease act 35) is compulsory for only high-risk herds that have been confirmed, or suspected of infection using RBT and CFT serological tests. A previous study on brucellosis among slaughter cattle observed a seroprevalence rate of 1.5% in an abattoir in the KwaZulu Natal province, and this abattoir-based study was conducted on cattle over three decades ago in the country (Bishop, 1984). In the Eastern Cape province, a 9.2% prevalence of *B. abortus* (of which 0.8% *B. abortus* S19 vaccine strain) was isolated from cattle, 2.9% *B. melitensis* from sheep and 6.3% *B. melitensis* from goats using different samples (blood, milk and lymph nodes) followed by species confirmation using PCR (Caine et al., 2017).

5.3 Materials and Methods

5.3.1 Study area, Study design and Abattoirs samples

The study area was the Gauteng Province (GP) of South Africa. GP is the smallest province in South Africa with approximately 1.5% (surface area of 1,219,602 km²) of the land area, yet it remains the densest province and houses approximately 23.7% of the country's population.

The province is strategic in terms of governance, economy and level of urbanization and stretches from 22°S to 35°S and from 17°E to 33°E. Although the estimated number of cattle in the Gauteng province at May 2018 was 246,395, it is known that a large number of cattle from other provinces may be moving to Gauteng on regular basis (DAFF., 2018).

A cross sectional study was conducted to determine the prevalence of brucellosis and detect, isolate and characterize the *Brucella* spp. in cattle slaughtered at the Gauteng province abattoirs from 2016 to 2018.

Fourteen abattoirs in the Gauteng Province (Figure 5.1), which were operational and consented to participate in the study, were randomly selected. These abattoirs were categorized into high throughput (n = 7) and low throughput (n = 7) facilities. While some operated as multi-species abattoir, others were mono-species during the study. These abattoirs received animals from different municipalities and communities within the province.

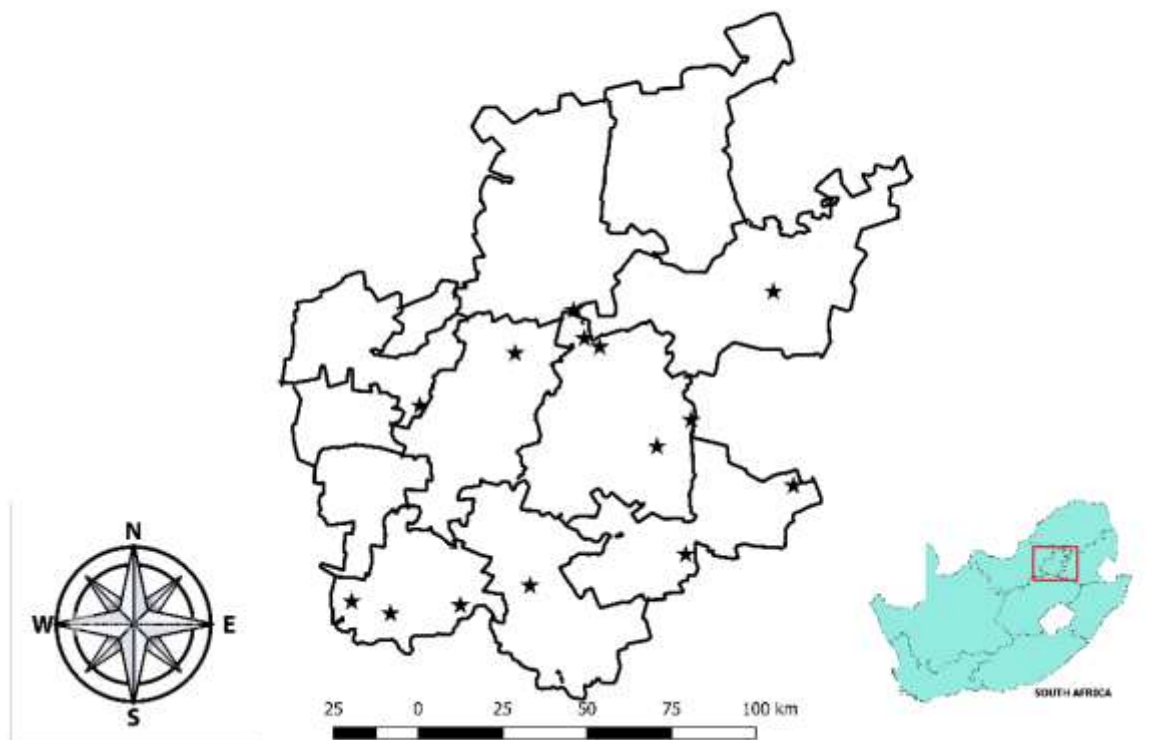


Figure 5-1: Locations of abattoirs sampled in the Gauteng Province of South Africa.

5.3.2 Sample size and collection

In the cross-sectional study, a systematic simple random sampling method was used to determine the sample size and power. Sample size was estimated at 50.0% expected frequency, with 5.0% acceptable margin of error and a design effect of 1 and clusters equals

to 1 in Epi-Info 7 version 10. At a 99.0% confidence level, the sample size of 200 animals was achieved. Only those farm sources with history of animals with herds having ten or more animals were included in the study. For all selected animals, blood samples were taken as described below.

Unclotted blood were collected from 200 cattle at the point of slaughter using a sterile 50ml cup; and approximately 5ml of the blood was aliquoted into a yellow-capped vacutainer tube. Corresponding tissue samples (lymph nodes, spleen and liver) were collected from each of the cattle. The lymph nodes of each animal were pooled and comprised the retropharyngeal, parotid, submandibular and mesenteric lymph nodes. The excised tissues were processed according to set laboratory protocols in a bio-safety laboratory level 2 plus. The homogenized tissues were used for DNA extraction and bacterial isolation. Demographic data were collected for each of the animal sampled, and these included, the animal species, breed, sex, age, the farm source of the animals and the abattoir where the animal was slaughtered. The age of the animals was determined using the dental formula during the inspection of the head, and thus animals were classified as an adult when two permanent incisors teeth are present or as young when the permanent teeth have not erupted (Eubanks, 2012). The vaccination status of the animals could not be ascertained.

5.3.3 Serological test methods on serum samples

Serological procedures were carried out using three different procedures including the Rose Bengal test (RBT), the complement fixation test (CFT) and the indirect enzyme-linked immunosorbent assay (iELISA). Rose Bengal test was conducted using 50µl volume of commercial IDEXX *Brucella* antigen (Switzerland) stained with Rose Bengal stain that was mixed with an equal volume of 50µl of test serum. The mixture was agitated gently for four minutes at room temperature on a rocker. Agglutination was read after four minutes and any visible agglutination was regarded as positive for brucellosis based on a standardized positive control (OIE, 2009). The diagnostic sensitivity and specificity for the RBT have earlier been confirmed to be 100% and 75%, based on previous validation studies (Nielsen et al., 2005, Stemshorn et al., 1985).

Furthermore, CFT was performed on the cattle serum samples at the Agriculture Research Council- Onderstepoort Veterinary Research laboratory, a South African National Accreditation System (SANAS)-accredited laboratory for bacteriology in South Africa using the OIE protocol (OIE, 2009). The cut-off value for this test was ≥ 30 IU/ml or more as an indication of infection and the obtained values were compared to the positive and negative controls.

Using the iELISA, commercial bovine IDEXX brucellosis serum X2 ELISA Test kit from Pourquier®, IDEXX, Switzerland) cattle sera were according to the manufacturer's instruction. The cut-off value for determination of antibody-positive status in cattle recommended by IDEXX is 80%.

5.3.4 Bacterial isolation from tissue samples

Homogenate (200 µl) from each tissue (lymph nodes, spleen and liver) was inoculated onto Farrell's and modified CITA media respectively and incubated at 37°C with 5.0% CO₂. Plates were observed for bacterial colony growth for ten days. *Brucella* organisms were identified presumptively by morphology using the Stamp's modified Ziehl-Neelsen staining method (OIE, 2009). Morphologically identified *Brucella* colonies were purified by sub-culturing on the media. Mixed or contaminated cultures were subjected to serial dilution 1:1000 using buffered peptone water and inoculated onto the media until purified colonies were obtained. Pure *Brucella* cultures from bovine were biotyped according to the methods described by Ribeiro and Herr (1990). Suspected *Brucella* cultures were again identified presumptively by morphological methods using Stamp's test. The *Brucella* isolates were later grown on sheep blood agar and biochemical tests were conducted as described by Ribeiro and Herr (1990) and OIE (2009).

5.3.5 Molecular detection

Genomic DNA was extracted from cultures of each homogenized tissue (lymph nodes, spleen and liver) and thereafter, the cells of *Brucella* cultures were purified using the set protocol according to Isolate II Genomic DNA kit by Bioline (South Africa). DNA detection and *Brucella* isolation were performed on all animals, regardless of their serological status. Genus-specific 16S-23S rRNA interspacer region (ITS) PCR was used to amplify *Brucella* region using *B. abortus* strain 544 (REF 544) and *B. melitensis* Rev 1 as positive controls. *Brucella* DNA was detected in tissue samples (lymph nodes, spleen and liver) of the slaughtered animals using the ITS-PCR assay as described by (Keid et al., 2007) that amplify a 214 bp fragment using primers (ITS66: ACATAGATCGCAGGCCAGTCA and ITS279: AGATACCGACGCAAACGCTAC). Primers were used at a final concentration of 0.2 µM with 1xDreamTaq Green PCR Master Mix (ThermoFisher Scientific, South Africa) and 2 µl DNA in a 15 µl PCR reaction. The PCR cycling condition consisted of 95°C for 3 minutes, followed by 35 cycles at 95°C for 1 minute, 60°C for 2 minutes, 72°C for 2 minutes and a final extension at 72°C for 5 minutes. PCR products were analysed by electrophoresis using a 2.0% agarose gel stained with ethidium bromide and viewed under UV light.

AMOS PCR: The multiplex AMOS PCR assay that identifies and differentiates *B. abortus*, *B. melitensis*, *B. ovis* and *B. suis* was conducted as described (Bricker and Halling, 1994, Weiner et al., 2011) using DNA extraction from cultures. Four species-specific forward primers were used at a final concentration of 0.1 μM with 0.2 μM reverse primer *IS711* (Table 1) with 1x MyTaq™ Red PCR Mix (Bioline, South Africa) and 2 μl of template DNA in 25 μl PCR reaction. PCR cycling condition was initial denaturation at 95°C for 5 minutes followed by 35 cycles at 95°C for 1 minute, 55.5°C for 2 minutes, 72°C for 2 minutes and a final extension step at 72°C for 10 minutes. PCR products were analyzed by electrophoresis using a 2.0% agarose gel stained with ethidium bromide and viewed under UV light.

Table 5.1: Sequences and characteristics of the oligonucleotide primers used for different *Brucella* species in the AMOS PCR assay.

PCR Name	Primer name	Sequence (5' - 3')	DNA Targets	Amplicon (bp)	Concentration (μM)	Reference
AMOS	<i>B. abortus</i>	GAC GAA CGG AAT TTT TCC AAT CCC	<i>IS711</i>	498	0.1	(Bricker et al., 2003, Bricker and Halling, 1994)
	<i>B. melitensis</i>	AAA TCG CGT CCT TGC TGG TCT GA		731	0.1	
	<i>B. ovis</i>	CGG GTT CTG GCA CCA TCG TCG GG		976	0.1	
	<i>B. suis</i>	GCG CGG TTT TCT GAA GGT GGT TCA		285	0.1	
	<i>IS711</i>	TGC CGA TCA CTT AAG GGC CTT CAT			0.2	

Bruce-ladder: A multiplex Bruce-ladder PCR assay to identify and differentiate between vaccine strains and field isolates of *Brucella* spp. was conducted as described (García-Yoldi et al., 2006, Lopez-Goñi et al., 2008, Weiner et al., 2011) (Table 5.2). Eight species-specific forward and reverse primers were used at a final concentration of 6.25 μM with 1x MyTaq™ Red PCR Mix (Bioline, South Africa) and 2 μl of template DNA in a 25 μl PCR reaction. The PCR cycling condition included an initial denaturation cycle at 95°C for 5 minutes followed by 25 cycles at 95°C for 30 seconds, at 64°C for 45 seconds, and at 72°C for 3 minutes and a final extension step at 72 °C for 10 minutes. PCR products were analyzed by electrophoresis using a 2.0% agarose gel stained with ethidium bromide and viewed under UV light.

Table 5.2: Sequences and characteristics of the Bruce-ladder PCR assay primers used in the study.

PCR Name	Primer name	Sequence (5' - 3')	DNA Targets	Amplicon (bp)	Concentration (μM)	Reference
Bruce-ladder	BMEI0998f BMEI0997r	ATC CTA TTG CCC CGA TAA GG GCT TCG CAT TTT CAC TGT AGC	<i>wboA</i>	1682	6.25	(Lopez-Goñi et al., 2008)
	BMEI0535f BMEI0536r	GCG CAT TCT TCG GTT ATG AA CGC AGG CGA AAA CAG CTA TAA	<i>bp26</i>	450	6.25	
	BMEI0843f BMEI0844r	TTT ACA CAG GCA ATC CAG CA GCG TCC AGT TGT TGT TGA TG	<i>omp31</i>	1071	6.25	
	BMEI1436f BMEI1435r	ACG CAG ACG ACC TTC GGT AT TTT ATC CAT CGC CCT GTC AC	Deacetylase	794	6.25	
	BMEI0428f BMEI0428r	GCC GCT ATT ATG TGG ACT GG AAT GAC TTC ACG GTC GTTCG	<i>eryC</i>	587	6.25	
	BR0953f BR0953r	GGA ACA CTA CGC CAC CTT GT GAT GGA GCA AAC GCT GAA G	ABC Transporter	272	6.25	
	BMEI0752f BMEI0752r	CAG GCA AAC CCT CAG AAG C GAT GTG GTA ACG CAC ACC AA	<i>rpsL</i>	218	6.25	
	BMEI0987f BMEI0987r	CGC AGA CAG TGA CCA TCA AA GTA TTC AGC CCC CGT TAC CT	CRP Regulator	152	6.25	

5.3.6 Statistical analysis

Data collected was managed using the Microsoft Excel version 2007. The R software (RCoreTeam, 2013) was used to analyze the data and to conduct descriptive analysis, and Epi-Info 7 version 10 was used to conduct analyses of frequency with 95% confidence interval, and a 2 x 2 table analysis for odd ratio, chi-square test as well as for plotting of charts. Herd prevalence was determined by the number of positive herds divided by the total number of herds included in the study. Animal prevalence was determined by the number of positive animals divided by the total number of animals sampled.

5.3.7 Ethical approval

Ethics approval for the study was obtained from ARC-OVI (Agricultural Research Council – Onderstepoort Veterinary Institute) Animals Ethics Committee (AEC12-16), University of Pretoria Animal Use and Care Committee (V089-16). Section 20 approval was granted according to Act 35 of 1984 by the Directorate of Animal Health, South Africa.

5.4 Results

Out of the cattle population sampled, 57.5% (115/200) were from HT abattoirs, while 42.5% (85/200) originated from LT abattoirs. Of the 200 heads of cattle, 41.0% (82/200) were female while 59.0% (118/200) were male. The distribution of the cattle stratified by age was 92.0% (184/200) for adult and 8.0% (16/200) for young cattle; the distribution stratified by breed was Bonsmara, 69.5% (139/200), Nguni, 16.5% (33/200), Brahman, 5.5% (11/200), Jersey, 5.0% (10/200) and Holstein, 3.5% (7/200).

Among the 200 cattle tested, 11.0% (22/200, 95% CI= 6.69-15.75) were positive on RBT, 5.5% (11/200, 95%CI=2.78-9.63) were positive on iELISA while on CFT, 18.2% (4/22) were positive from the RBT. Of the iELISA-positives, 50.0% (11/22) were from the RBT-positive cattle, yielding an estimated seroprevalence of 5.5% (RBT and confirmed by iELISA). The cattle herd seroprevalence was 85.0% (12/14) on RBT and 75.0% (10/14) on PCR.

The serology positivity, PCR detection rates and isolation rates among the cattle population according to sex, age and the type of abattoirs sampled with the measure of association is shown in Table 5.3.

The distribution of cattle that tested seropositive by RBT and confirmed with ELISA according to breed was as follows: Bonsmara, 3.6% (5/139); Nguni, 9.1% (3/33); Holstein, 28.6% (2/7); Brahman, 9.1% (1/11) and Jersey, 0.0% (0/10). The differences were not statistically significant ($P>0.05$).

Brucella DNA detection rate from the cattle tissue by ITS-PCR was 12.5% (25/200). Figure 5.2 illustrates the gel electrophoresis picture from the ITS-PCR conducted on some of the isolates. Table 5.3 illustrates the positivity with ITS-PCR among the cattle population according to sex, age and the type of abattoirs sampled. The measure of association between the sex ($P=0.01$) and abattoir types ($P=0.00$) with *Brucella* DNA detection rate by PCR were statistically significant. Of the 25 ITS-PCR positives, 56.0% (14/25) were from seropositive cattle (RBT or ELISA), while 44.0% (11/25) were from seronegative cattle.

Table 5.3: Prevalence and risk of *Brucella* spp. from abattoirs samples by seropositivity, 16S-23S rRNA interspacer region (ITS) PCR and the isolation rate.

Animal demography	Total	Serology positives ^a (%)	^b OR (95% ^c CI)	Chi (X^2)	P-value
Seropositivity to <i>Brucella</i> spp. stratified by sex, age of animals and type of abattoirs sampled					
Sex					
Female	82	16 (19.5)	4.5 (1.67-12.13)	8.9	0.00
Male	118	6 (5.1)			
Age					
Adult	184	18 (9.8)	0.3 (0.09-1.11)	2.1	0.15
Young	16	4 (25.0)			
Abattoir type					
High throughput	115	4 (3.5)	0.1 (0.04-0.41)	13.9	0.00
Low throughput	85	18 (21.3)			
Animals positive by 16S-23S rRNA interspacer region (ITS) ^dPCR stratified by sex, age and type of abattoirs sampled					
Sex					
Female	82	17 (20.7)	3.60 (1.47-8.80)	7.4	0.01
Male	118	8 (6.8)			
Age					
Adult	184	22 (12.0)	0.59 (0.15-2.23)	0.2	0.69
Young	16	3 (18.8)			
Abattoir type					
High throughput	115	6 (5.2)	0.19 (0.07-0.51)	11.4	0.00
Low throughput	85	19 (22.4)			
Isolation rate by bacteriological method stratified by sex, age of animals and type of abattoirs sampled					
Sex					
Female	82	8 (9.8)	4.14 (1.06-16.12)	3.6	0.06
Male	118	3 (2.5)			
Age					
Adult	184	9 (4.9)	0.36 (0.7-1.83)	0.5	0.48
Young	16	2 (12.2)			
Abattoir type					
High throughput	115	1 (0.9)	0.06 (0.01-0.52)	9.2	0.00
Low throughput	85	10 (11.7)			

a = percentage, *b* = odds ratio, *c*=confidence interval, *d* = polymerase chain reaction.

With the use of the molecular method, the distribution of positivity according to the breed was as follows: Bonsmara 10.8% (15/139), Nguni 12.1% (4/33), Holstein 42.8% (3/7), Brahman 27.3% (3/11) and Jersey 0.0% (0/10). The differences were not statistically significant ($P>0.05$).

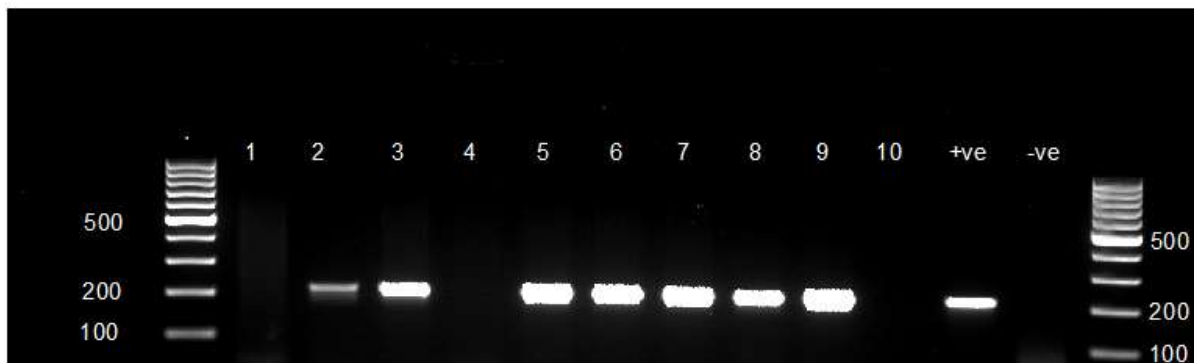


Figure 5-2: Gel electrophoresis of products from ITS-PCR amplification of the 16-23SrRNA gene using primers ITS66F and ITS 279R. **Lanes 2, 3, 5, 6, 7, 8, 9** show amplification product of 214bp in length and the last two lanes shows the positive and negative controls.

The isolation rate of *Brucella* spp. was 5.5% (11/200). Of the cattle tested with bacteriological methods, the distribution of isolation positivity according to the breed of the animals was as follows: Bonsmara, 4.3% (6/139), Nguni 3.0% (1/33), Holstein 42.8% (3/7), Brahman 9.1% (1/11) and Jersey 0.0% (0/10). The differences were not statistically significant ($P > 0.05$).

Of the 11 confirmed *Brucella* isolates from cattle by the “gold standard” method of culture and isolation, 63.6% (7/11) were from the confirmed iELISA-positive cattle. The remaining 4 (36.4%) iELISA-positives could not be isolated, but from the other four isolates, three were from RBT-positive cattle and one was from a seronegative cow. AMOS-PCR assay characterized the seven isolates from the iELISA-positives as *B. melitensis* (four isolates) and *B. abortus* (three isolates). The remaining four isolates, three of which were RBT-positives, were classified as *B. melitensis* (two) and *B. abortus* (one); while the only single isolate that was negative on serology was characterized as *B. abortus*. In total, the AMOS-PCR characterized the 11 isolates as six *B. melitensis* and five *B. abortus*. One out of these isolates had amplification for both *B. abortus* and *B. melitensis* (Figure 5.3). All isolates were differentiated from the vaccine strains using the Bruce-Ladder PCR assay (Figure 5.4). Of the seven *Brucella* isolates from the iELISA-positive cattle, five isolates were biotyped using biochemical tests of which two were determined to be *B. melitensis* bv 3, one as *B. melitensis* bv 2 and the remaining two as *B. abortus* bv 1.

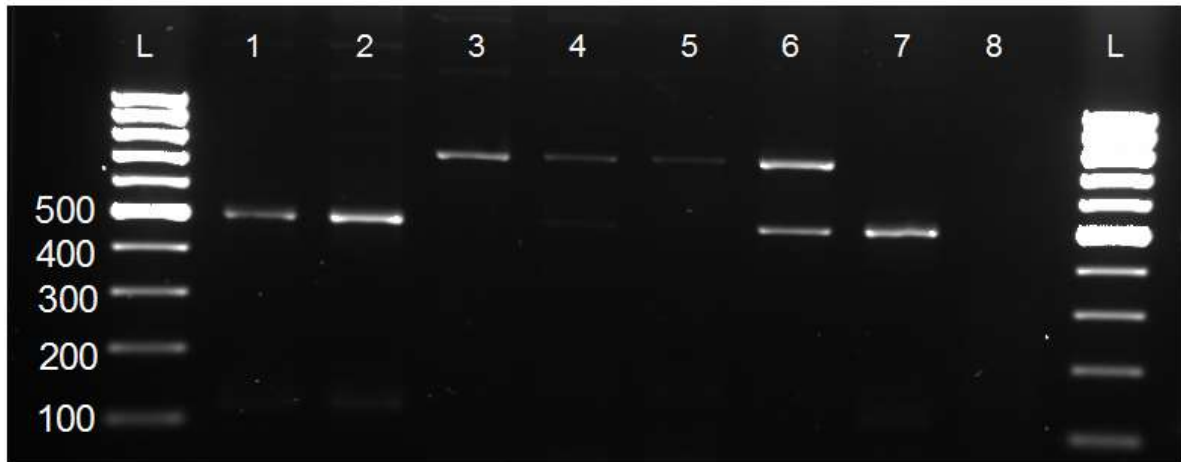


Figure 5-3: Gel electrophoresis of products from AMOS-PCR of the *IS711* gene using species-specific primers. **Lanes 1 and 2** show amplification products of 498bp in length for *B. abortus* and **Lanes 3 to 6** show amplification products of 710bp for *B. melitensis*, while **Lanes 4 and 6** show double amplification as a result of mixed infection of both *B. abortus* and *B. melitensis*, in the samples. **Lanes 7 and 8** show the positive and negative control, **lane L** shows 100bp DNA ladder (Invitrogen, ThermoFisher® scientific, South Africa).

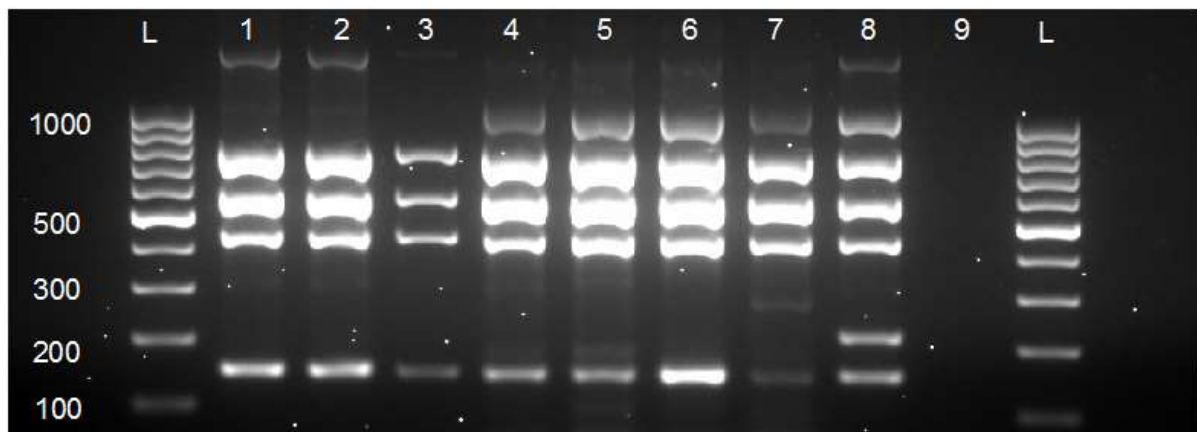


Figure 5-4: Gel electrophoresis of Bruce-Ladder PCR amplification product using species specific primers. **Lanes 1 and 2** show amplification product of *B. abortus*, **lane 3** is *B. abortus* strain 544 (control), **lanes 4 and 5** show amplification for *B. melitensis* (cattle) and **lanes 6 and 7** show amplification for *B. melitensis* (sheep), **lanes 8 and 9** show REV 1 positive and negative controls respectively, **lane L** shows 100bp DNA ladder (Invitrogen, ThermoFisher® scientific, South Africa).

Out of the tissue samples from the 200 slaughtered cattle tested using the ITS-PCR for the detection of *Brucella* DNA, the frequencies of detection were 11.5% (23/200, 95%CI=7.43-16.6) from the lymph nodes, 7.5% (15/200, 95%CI=4.26-12.1) from the spleen and 7.0% (14/200, 95%CI=3.88-11.5) from the liver. The detection rates from the three tissue samples among the 25 ITS-PCR-positive cattle are lymph nodes 92.0% (23/25), 64.0% (16/25) and 56.0% (14/25) for the lymph nodes, spleen and liver respectively.

Of the 25 ITS-PCR positive cattle, the frequency of detection was 68.0% (17/25, 95%CI=46.5-85.1) in the females, and 32.0% (8/25, 95%CI=14.9-53.5) in the males. Of the 25 ITS-PCR

positive cattle, the distribution by age classification was 88.0% (22/25, 95%CI=68.8-97.4) for adults, and 12.0% (3/25, 95%CI=2.5-31.2) for young cattle; and by abattoir types was 24.0% (6/25, 95%CI=9.36-45.1) for HT abattoirs and 76.0% (19/25, 95%CI=54.9-90.6) for LT abattoir.

Among the 23, 15 and 14 ITS-PCR-positive lymph nodes, spleen and liver tissues respectively, the frequency of isolation was 26.1% (6/23) from the lymph nodes, 40.0% (6/15) from the spleen and 42.8% (6/14) from the liver samples. Of the total 25 PCR-positive cattle, the frequency of *Brucella* spp. isolation was 44.0% (11/25, 95%CI=24.4-65.1). The isolation rate from all the three tissue samples among the 11 culture-positive cattle was 26.1% (6/23) for each tissue.

From the 11 *Brucella* culture-positive cattle, the frequency of isolation of *Brucella* spp. by sex was 72.7% (8/11, 95%CI=39.0-94.0) in the females and 27.3% (3/11, 95%CI=6.02-61.0) in the males and by age was 81.8% (9/11, 95%CI=48.2-97.7) in adults and 18.2% (2/11, 95%CI=2.28-51.8) in the young cattle. The frequency of isolation was 9.1% (1/11, 95%CI=0.23-41.3) and 90.9% (10/11, 95%CI=58.7-99.9) in the HT and LT abattoirs respectively.

5.5 Discussion

This study has shown that the prevalence of brucellosis among the slaughter cattle at the Gauteng abattoirs is at 5.5% using the gold standard of isolation. The estimated seroprevalence was 5.5% (RBT and iELISA). Our finding of 11.0% seroprevalence of brucellosis by the RBT is higher than a seroprevalence rate of 3.95% on RBT reported for brucellosis in slaughter cattle in Pakistan, and the 5.5% seroprevalence rate detected by the iELISA in our study is comparable to the 5.9% seropositivity reported, also using iELISA in the Pakistani study (Shafee et al., 2012). In comparison to other regions of the Southern African Development Countries (SADC), the estimated seroprevalence of 5.5% in this study is lower than the range of 14.1% to 28.1% reported in a study in Zambia (Muma et al., 2006). Chimana et al. (2010) reported a seroprevalence ranging from 7.9% to 18.7% of brucellosis among cattle population in Zambia which is comparable to the result observed in this study. Although the animals studied in Zambia were not from abattoir facilities, however, this underscores the endemicity of the disease in countries around the SADC region.

In South Africa, testing scheme for bovine brucellosis (established under section 10 of the Animal Disease act 35) is compulsory for only high-risk herds that have been confirmed, or suspected of infection using RBT and CFT serological tests. Entering the brucellosis testing scheme is voluntary for all other bovine herds and livestock owners. Due to the predominant

voluntary nature of the brucellosis testing scheme in South Africa, known and unknown brucellosis infected cattle can be slaughtered at abattoirs. The vaccination status of the slaughtered animals was unknown. Vaccination of three to eight months heifers with *B. abortus* S19 is compulsory. However, the percentage of cattle vaccinated with S19 is unknown as the vaccine producer of S19 (Onderstepoort Biological Product) only indicate overall total vaccine sold. There was also a period when *B. abortus* S19 vaccine was unavailable (DAFF, 2017). There is no compulsory testing to monitor *B. melitensis* in sheep and goats in South Africa (DAFF, 2017). *B. melitensis* has been reported in South Africa in sheep and goats, but surveillance in these species is conducted only when human brucellosis is detected and associated with sheep and goats (Emslie and Nel, 2002). This abattoir survey provided useful baseline data on the prevalence of brucellosis in the tested animal population within a region or the country.

The detection of *B. abortus* bv 1 among the isolates from our study did not come as a surprise because it has been documented to be the common species and biotype infecting the cattle population in South Africa (Coetzer et al., 1994, Gradwell, 1977), and in other countries especially in the Southern African region and the Caribbean (Fosgate et al., 2002, Matope et al., 2009, Muendo et al., 2012). The isolation of *B. melitensis* from cattle in this study is however significant because it is considered the first documentation of *B. melitensis* in the cattle population in South Africa (Kolo et al., 2018). The occurrence of *B. melitensis* in cattle in this study may be as a result of rearing cattle together with sheep or goats on the same farm or sharing of grazing land with sheep or goats (Radostits et al., 2006, Verger et al., 1989). This has huge implication to infection of other cattle in the herd and most importantly spill over to other farms and other species around that geographical areas (Godfroid et al., 2014). The detection of both *B. melitensis* and *B. abortus* is been reported for the first time in cattle in South Africa. *Brucella melitensis* bv 2 and 3 have never been isolated in the cattle population country. This has significant implications for brucellosis control in South Africa as the brucellosis scheme only focus on testing bovine high risk herds. However, this study report *B. melitensis* bv 2 and 3 to be present in cattle and therefore, there is a high probability of its presence in the sheep and goat populations. *Brucella melitensis* was isolated and reported in humans in the Western Cape province in 2015 (Wojno et al., 2016). Furthermore Caine et al. (2017) detected *B. melitensis* from the tissues and blood samples of sheep at abattoirs in the Eastern Cape province. Biotyping was not done with the *B. melitensis* from the Western and Eastern Cape studies.

In our study, AMOS-PCR indicated a mixed infection of *B. abortus* and *B. melitensis* (Figure 5.3) in an impure culture, but Bruce-ladder can differentiate a mixed infection of both organisms since it will only show the profile of *B. melitensis*. Mixed infection is plausible if

morphological identical *Brucella* colonies of different species grow on the same plate. In future studies, the impure culture could be purified by picking multiple colonies resulting from the same animal and speciating the pure cultures using PCR and/or biotyping.

Brucellosis is a herd disease and from our study, the estimated herd seroprevalence was 85.0% (12/14) by serology. A cross-sectional study conducted between 2015 and 2016 in Gauteng province using RBT and CFT detected bovine brucellosis herd prevalence of 13.7% (24/175) (Gauteng Province Veterinary Services Annual Report 2015-2016)(GDARD, 2016). In Gauteng province the government reported 6.3% brucellosis infected commercial herd and 1.9% communal herd in 2017-2018 (GDARD, 2018). The latter brucellosis prevalence was obtained on mainly high-risk cattle tested as part of the bovine brucellosis scheme in South Africa and are therefore there is bias in the reporting. The higher herd prevalence in our study should be interpreted with caution due to the small sample size.

Using PCR for prevalence estimation, a herd prevalence of 75.0% (10/14) was determined in cattle. The ITS-PCR is a good option as a screening tool for *Brucella* DNA as the PCR is capable of detecting very small amount of DNA in the tissue samples even if as little as 3.8 fg of *Brucella* DNA mixed with 450 ng of host DNA (Keid et al., 2007). This is significant because the application of PCR to detect *Brucella* DNA in animal tissues can be used to diagnose brucellosis in immune-compromised animals that are unable to seroconvert following exposure and infection, and in animals where the *Brucella* organism, which is an intra-cellular organism, is localized in the tissues, such that serology may not be able to diagnose the infection from the serum samples (Radostits et al., 2006). However, the ITS-PCR sensitivity and specificity should be validated in South Africa especially to ensure the specificity of this PCR, as it could react with *Brucella*-like organisms in this region that could not be tested in the initial validation by Keid et al. (2007). The PCR method used in our study has proven to be fast, safe and does not require specialized laboratory as required in the bacteriological methods. Based on the detection rate of 92.0% in the lymph node tissues in our study, we recommend the pooling of the lymph nodes initially to assay for *Brucella* DNA. The strategic application of bacteriology, serology and PCR was shown to result in increased detection frequency of brucellosis in cattle.

5.6 Recommendations

This study has observed that no sound seroprevalence estimates can be drawn unless a sound sampling plan can be implemented. It is also recommended that abattoir-based study be conducted at provincial and national levels, to ascertain the frequency of brucellosis detection in all the nine provinces of the country. A testing strategy based on RBT and iELISA

should be used in series which is a sound testing strategy. This will provide baseline data for policy makers to proffer solutions and interventions to mitigate the risk of economic losses to livestock in the country and to mitigate the public health impact of the disease in the human population.

5.7 Conclusions

In conclusion, this study has provided evidence-based data that *B. melitensis* may be circulating in the cattle population in South Africa. *B. melitensis* bv 2 and 3 have never been isolated in the cattle population in the country. This suggests a potential risk to consumers of unpasteurized milk or milk products, and meat and meat products. The study also provided a current data on the prevalence (isolation, serology and PCR) of brucellosis in slaughter cattle in Gauteng province, South Africa. The seropositivity observed in slaughter cattle in this study emphasizes the importance of using abattoirs for passive and active surveillance of diseases of public health and economic importance. This study has also confirmed that as a diagnostic strategy, it is imperative to institute more than one diagnostic method or test for the diagnosis of brucellosis in animals.

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Chapter 6

Seroprevalence and characterization of *Brucella* spp. in slaughtered sheep and pigs in abattoirs in Gauteng province, South Africa

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6.1 Abstract

In South Africa, *B. melitensis* is a controlled disease in sheep and goats and the occasional outbreak has been reported and controlled through test and slaughter. *Brucella suis* has not been reported in South Africa. There is no brucellosis testing scheme for brucellosis in sheep, goats and pigs in South Africa. In 2015, *B. melitensis* has been reported in humans in the Western Province of South Africa. Seroprevalence and characterization of *Brucella* spp. were investigated using serological, molecular and bacteriological methods in across-sectional simple random sampling method of 142 slaughter animals (57 sheep and 85 pigs) in the Gauteng Province. The RBT and iELISA screening revealed a seroprevalence of 0.0% in sheep and pigs while the iELISA confirmed 1.8% (1 of 57) for *B. ovis* in the sheep sampled. *Brucella* DNA from both seronegative sheep and pigs was detected in 93.0% (53 of 57) of the sheep and 27.1% (23 of 85) of the pigs using the genus specific 16-23 ribosomal DNA interspacer region (ITS) PCR assay on tissue samples. ITS-PCR is not an OIE recommended PCR and not validated in South Africa. The ITS-PCR results could not be confirmed with OIE recommended PCR assays and therefore the pig samples are negative for brucellosis.

In the sheep, AMOS-PCR characterized 25 isolates as 18 *B. melitensis* and 7 as *B. ovis* (which included the one *B. ovis* seropositive sheep). The pig samples were negative for brucellosis as it did not amplify using the genus specific *bcs*p31 region real-time PCR and AMOS-PCR. The brucellosis scheme in South Africa is biased toward the cattle population while sheep, goats and pigs have not received much attention. The 31.6% (18/57) *B. melitensis* AMOS-

PCR positive from the seronegative sheep samples slaughtered at abattoirs is a major concern. Due to the small sample size a thorough investigation needs to be established to investigate brucellosis in sheep and pigs in South Africa to establish baseline prevalence data as well as to validate ITS-PCR for sheep, goats but especially pigs.

6.2 Introduction

Brucellosis is a neglected zoonotic disease that affects domestic animals and humans; with huge food safety implication and economic significance. Brucellosis in sheep is caused mainly by *B. ovis* and *B. melitensis* respectively (Alton, 1990b, McFarlane et al., 1952, Olsen and Palmer, 2014, Zammit, 1905). *Brucella melitensis* causes infection in sheep, goats and occasionally in humans (Corbel, 2006, Godfroid et al., 2005, Radostits et al., 2006). *Brucella ovis* does not cause disease in humans, but its economic impact on sheep is highly significant (Godfroid et al., 2005, Radostits et al., 2006). Brucellosis in pigs is a chronic disease characterized by orchitis and impotence in the males, abortion in the females and high mortality in the piglets (Alton, 1990a, Godfroid et al., 2013, Hutchings, 1950). *Brucella suis* causes swine brucellosis (Alton, 1990a, Godfroid et al., 2010, Hutchings, 1950, Traum, 1914).

Historically, *B. melitensis* was isolated from the milk of goats by Zammit (1905). *Brucella suis* was described by Traum (1914), and eventually in 1952, *B. ovis* was isolated from ram in New Zealand by McFarlane et al. (1952). *Brucella melitensis* has three biovars (bv. 1, 2, 3) with host preference for sheep and goats that are distributed in various geographical regions (OIE, 2016, Radostits et al., 2006). Five biovars of *B. suis* are known of which bv 1, 2 and 3 are significant to domestic and wild pigs (Cvetnić et al., 2009, Olsen and Palmer, 2014, Radostits et al., 2006), while *B. suis* bv 4 and 5 are implicated in causing diseases in wildlife (Forbes and Tessaro, 1993, Godfroid et al., 2010, Radostits et al., 2006). The occurrence of brucellosis in sheep caused by *B. ovis* is reported in many sheep producing countries around the world (Radostits et al., 2006).

Brucella ovis is enzootic in countries like Australia, New Zealand, Central Asia, South Africa, South America and the United States of America (Godfroid et al., 2005, Radostits et al., 2006). *Brucella melitensis* in sheep and goats is minimally distributed (FAO, 2009, Radostits et al., 2006) and primarily distributed in the Mediterranean regions and southern Europe areas (Alton, 1990b, Corbel, 2006, Pappas et al., 2006). Other places enzootic to this disease include; central and West Asia, South America and Africa (FAO, 2009, Godfroid et al., 2005, Radostits et al., 2006). The occurrence of porcine brucellosis influences mainly pig producing countries in Africa, Europe, South America, Central Asia, Australia, India, and the Pacific

islands (Fretin et al., 2013, Olsen and Palmer, 2014, Radostits et al., 2006, Szulowski et al., 2013).

An estimation of over 7,309,000 tons and 2,926,000 tons of sheep and pigs are slaughtered as of August 2015 in South Africa abattoirs annually (DAFF, 2016). These abattoir facilities can also be used for monitoring disease control policies, detection of newly introduced disease agents, and for the assessment of intervention programmes; such as brucellosis vaccination, and most importantly, abattoir survey may also allow for early intervention to mitigate the epidemic loss of animals (Alton et al., 2015, Fasina et al., 2015, Kaneene et al., 2006). Abattoir workers can be at risk of exposure to *Brucella*-infected animals at abattoirs (Corbel, 2006).

Brucellosis is a controlled disease in South Africa but mainly focus on cattle caused by *B. abortus* as *B. melitensis* outbreaks in sheep and goats have occurred in South Africa and most outbreaks was first detected in humans associated with the sheep and goats (Emslie and Nel, 2002, Reichel et al., 1996, Wojno et al., 2016). Simpson et al. (2018) determined the seroprevalence of caprine brucellosis in the Mnisi area in Limpopo province to be 0.17% in South Africa. In Namibia that borders South Africa, Madzingira and McCrindle (2015) reported a 0.14% ovine brucellosis seroprevalence over a period of three years and 0.00% seroprevalence of *B. melitensis* and *B. ovis* from sampled sheep. In contrast to these serological studies, a 2.9% and 6.3% *B. melitensis* was isolated from sheep and goats respectively at abattoirs in the Eastern Cape province of South Africa using blood, milk and lymph nodes samples and confirmed using AMOS PCR (Caine et al., 2017). This underscores the fact that direct detection of the *Brucellae* from slaughter animals' tissues can provides a sensitive diagnostic approach for brucellosis on either seropositive or seronegative animals. In this study, unclotted blood samples with corresponding tissue samples (lymph nodes, spleen and liver) from sheep and pigs were collected from eight abattoirs in the Gauteng province of South Africa and investigated for brucellosis using serological, molecular and bacteriological methods.

6.3 Materials and methods

6.3.1 Study area

The study area was the Gauteng Province of South Africa. The country stretches from 22°S to 35°S and from 17°E to 33°E, with a surface area of 1,219,602 km². The country has several distinct ecosystems and it is bounded by 2,798 km of coastline stretching along the South Atlantic and the Indian Oceans. In the north, its neighbours are countries of Namibia, Botswana, Zimbabwe and to the east and northwest by countries of Mozambique and

Swaziland. The estimated number of sheep and pigs in the Gauteng province at May 2018 was sheep 92,160 and pigs 156,264 (DAFF., 2018).

6.3.2 Study design

A cross sectional study was conducted to determine the prevalence of brucellosis and detect, isolate and characterize the *Brucella* spp. in sheep and pigs slaughtered at the Gauteng province abattoirs from April 2017 to April 2018.

6.3.3 Abattoirs sampled and sample size

Eight operational and consenting abattoirs in the Gauteng Province were randomly selected. A systematic simple random sampling method as determined using the Epi-Info 7 version 10 StatCalc for sample size and power, estimated the sample size at 50.0% expected frequency, with 5.0% acceptable margin of error, and with a design effect of 1 and clusters equals to 1; with a 99.0% confidence level, the sample size of 142 animals was achieved.

6.3.4 Sample collection

From the visited abattoirs, 142 animals (57 sheep and 85 pigs) were sampled and unclotted blood samples with corresponding tissue samples (lymph nodes, spleen and liver) were collected from each of the animals. These lymph nodes were selected on the bases that of animal carcasses from acute infection, the preferred tissues for cultures are those of the retico-endothelial system such as lymph nodes in the head, the spleen among others and in chronic stage in the liver (Bruce, 1887, Cheville et al., 1995, OIE, 2009, Palmer et al., 1996). DNA detection and *Brucella* isolation were performed on all animals, regardless of their serological status.

Unclotted blood samples were collected at the point of slaughter using a sterile 50ml cup; and approximately 5ml of the blood was aliquoted into a yellow capped vacutainer tube. The unclotted blood in the vacutainers were subjected to centrifugation at 3500 rpm for 2 minutes using the Eppendorf centrifuge 5810 R machine (Germany), and about 1.5ml of the separated serum was placed into a labeled 2ml Eppendorf tubes using a 1000 μ l pipette and stored in a -20°C freezer for use later.

Each tissue samples (lymph nodes, spleen and liver) from each identified and corresponding slaughter animal were stored in a -20°C freezer for further processing. Tissue samples from the sheep and pigs were stored and processed (serological tests, PCR and culturing) separately starting with the sheep samples and then followed by the pigs. The excised tissues (lymph nodes, spleen and liver) were processed according to set laboratory protocols in a bio-

safety laboratory level 2 plus at the Department of Veterinary Tropical Disease (DVTD), Faculty of Veterinary Sciences, University of Pretoria. Approximately 200mg of each tissue sample was cut and placed in a 1 ml of 10% PBS and homogenized with a Precellys 24 homogenizer (Bertin Technologies, France) at 5,200 rpm twice for 2 minutes. The homogenized tissues were used for DNA extraction and bacterial isolation. Demographic data were collected for each of the animal sampled, and these included, the animal species, breed, sex, age, the farm source of the animals and the abattoir where the animal was slaughtered. The age of the animals was determined using the dental formula during the inspection of the head, and thus was classified as an adult when two permanent incisors teeth are present or as young when the permanent teeth had not erupted (Eubanks, 2012). The vaccination status of the animals could not be ascertained.

6.3.5 Statistical analysis

Data collected was managed using the Microsoft Excel version 2007. The R software (RCoreTeam, 2013) was used to analyze the data and to conduct descriptive analysis, Epi-Info 7 version 10 was used to conduct analyses of frequency with 95% confidence interval, and a 2 x 2 table analysis for odd ratio, chi-square test as well as for plotting of charts. Herd prevalence was determined by the number of positive herds divided by the total number of herds animals were presented from. Animal prevalence was determined by the number of positive animals divided by the total number of animals sampled. The gold standard of diagnosis of brucellosis is culture and isolation, therefore positivity of animals in this study is based on isolation of *Brucella* spp. confirmed by PCR.

6.3.6 Serological test methods on serum samples

Rose Bengal test (RBT): was done using the 50µl volume of commercial IDEXX *Brucella* antigen (Switzerland) stained with Rose Bengal that was mixed with an equal volume of 50µl of test serum and the mixture was agitated gently for four minutes at room temperature on a rocker. Agglutination was read after four minutes and any visible agglutination was regarded as positive for brucellosis as compared with the positive control (OIE, 2009). The diagnostic sensitivity and specificity for the RBT were assumed to be 100% and 75%, based on previous validation studies respectively (Nielsen et al., 2005, Stemshorn et al., 1985)

Indirect enzyme-linked immunosorbent assay (iELISA): The commercial ovine and caprine IDEXX brucellosis serum X2 ELISA Test kit from Pourquier® (IDEXX, Switzerland) was used to test the sheep serum according to the manufacturer's instruction. The cut-off value for determination of antibody-positive status in sheep recommended by IDEXX is 120%. For the ovine iELISA, the commercial *B. ovis* antibody test kit from Pourquier® (IDEXX, Switzerland)

was used to detect antibodies to *B. ovis* according to the manufacturer's instructions, with a cut-off value of 120% for determining the antibody-positive status. The pig serum was tested using the brucellosis serum indirect multi-species antibody ELISA test kit by IDvet (France) according to the manufacturer's instructions with a cut-off value of 120% for determining the antibody-positive status. The diagnostic sensitivity and specificity for the iELISA were assumed to be 95.5% and 98.1% respectively based on previous validation studies (Nielsen et al., 2005).

6.3.7 Bacterial isolation from tissue samples

Homogenate (200 µl) from each tissue (lymph nodes, spleen and liver) was inoculated onto Farrell's and modified CITA media respectively and incubated at 37°C with 5.0% CO₂. Plates were observed for bacterial colony growth for ten days. Suspect *Brucella* organisms were identified presumptively at the SANAS-accredited ARC-OVR bacteriology laboratory by morphological methods using stamps test (OIE, 2009).

6.3.8 Molecular detection

Genomic DNA were extracted first from homogenized tissues each namely lymph nodes, spleen and liver and thereafter, from the inoculated tissue using the set protocol according to Isolate II Genomic DNA kit by Bioline (South Africa). Extracted DNA was labelled and placed in a -20°C freezer for storage.

Genus specific 16S-23S rRNA interspacer region (ITS) PCR was used to amplify *Brucella* region using *B. abortus* bv 1 strain (REF 544, BCCN R4) and *B. melitensis* Rev 1 (Onderstepoort Biological Products, South Africa) as positive controls. *Brucella* DNA was detected in tissue samples (lymph nodes, spleen and liver) of the slaughtered animals using the ITS-PCR assay as described by (Keid et al., 2007) that amplify a 214 bp fragment using primers (ITS66: ACATAGATCGCAGGCCAGTCA and ITS279: AGATACCGACGCAAACGCTAC). Primers were used at a final concentration of 0.2 µM with 1xDreamTaq Green PCR Master Mix (ThermoFisher Scientific, South Africa) and 2 µl DNA in a 15 µl PCR reaction. The PCR cycling condition consisted of 95°C for 3 minutes, followed by 35 cycles of 95°C for 1 minute, 60°C for 2 minutes, 72°C for 2 minutes and a final extension of 72°C for 5 minutes. PCR products were analysed by electrophoresis using a 2.0% agarose gel stained with ethidium bromide and viewed under UV light.

AMOS PCR: The multiplex AMOS PCR assay that identifies and differentiates *B. abortus*, *B. melitensis*, *B. ovis* and *B. suis* was conducted as described (Bricker and Halling, 1994, Weiner et al., 2011) using DNA extraction from sheep cultures. Four species-specific forward primers

were used at a final concentration of 0.1 μM with 0.2 μM reverse primer *IS711* (Table 5.1) with 1 x MyTaq™ Red PCR Mix (Bioline, South Africa) and 2 μl of template DNA in 25 μl PCR reaction. PCR cycling condition was initial denaturation at 95°C for 5 minutes followed by 35 cycles of 95°C for 1 minute, 55.5°C for 2 minutes, 72°C for 2 minutes and a final extension step at 72°C for 10 minutes. PCR products were analyzed by electrophoresis using a 2.0% agarose gel stained with ethidium bromide and viewed under UV light.

Table 6.1: Sequences and characteristics of the oligonucleotide primers used for different *Brucella* species in the AMOS PCR assay.

PCR Name	Primer name	Sequence (5'-3')	DNA Targets	Amplicon (bp)	Concentration (μM)	Reference
AMOS	<i>B. abortus</i>	GAC GAA CGG AAT TTT TCC AAT CCC	<i>IS711</i>	498	0.1	(Bricker et al., 2003, Bricker and Halling, 1994)
	<i>B. melitensis</i>	AAA TCG CGT CCT TGC TGG TCT GA		731	0.1	
	<i>B. ovis</i>	CGG GTT CTG GCA CCA TCG TCG GG		976	0.1	
	<i>B. suis</i>	GCG CGG TTT TCT GAA GGT GGT TCA		285	0.1	
	<i>IS711</i>	TGC CGA TCA CTT AAG GGC CTT CAT			0.2	

Bruce-ladder: A multiplex Bruce-ladder PCR assay to identify and differentiate between vaccine strains and *Brucella* field isolates. was conducted as described (García-Yoldi et al., 2006, Lopez-Goñi et al., 2008, Weiner et al., 2011) (Table 6.2). Eight species-specific forward and reverse primers were used at a final concentration of 6.25 μM with 1x MyTaq™ Red PCR Mix (Bioline, South Africa) and 2 μl of template DNA in a 25 μl PCR reaction. The PCR cycling condition included an initial denaturation cycle of 95°C for 5 minutes followed by 25 cycles at 95°C for 30 seconds, at 64°C for 45 seconds, and at 72°C for 3 minutes and a final extension step at 72 °C for 10 minutes. PCR products were analyzed by electrophoresis using a 2.0% agarose gel stained with ethidium bromide and viewed under UV light.

Table 6.2: Sequences and characteristics of the Bruce-ladder PCR assay primers used in the study.

PCR Name	Primer name	Sequence (5'-3')	DNA Targets	Amplicon (bp)	Concentration (μM)	Reference
Bruce-ladder	BMEI0998f	ATC CTA TTG CCC CGA TAA GG	<i>wboA</i>	1682	6.25	(Lopez-Goñi et al., 2008)
	BMEI0997r	GCT TCG CAT TTT CAC TGT AGC				
	BMEI0535f	GCG CAT TCT TCG GTT ATG AA	<i>bp26</i>	450	6.25	
	BMEI0536r	CGC AGG CGA AAA CAG CTA TAA				
	BMEI0843f	TTT ACA CAG GCA ATC CAG CA	<i>omp31</i>	1071	6.25	
	BMEI0844r	GCG TCC AGT TGT TGT TGA TG				
	BMEI1436r	ACG CAG ACG ACC TTC GGT AT	Deacetylase	794	6.25	
	BMEI1435r	TTT ATC CAT CGC CCT GTC AC				
	BMEI0428f	GCC GCT ATT ATG TGG ACT GG	<i>eryC</i>	587	6.25	
	BMEI0428r	AAT GAC TTC ACG GTC GTTCG				
	BR0953f	GGA ACA CTA CGC CAC CTT GT	ABC	272	6.25	
	BR0953r	GAT GGA GCA AAC GCT GAA G	Transporter			
BMEI0752f	CAG GCA AAC CCT CAG AAG C	<i>rpsL</i>	218	6.25		
BMEI0752r	GAT GTG GTA ACG CAC ACC AA					
BMEI0987f	CGC AGA CAG TGA CCA TCA AA	CRP	152	6.25		
BMEI0987r	GTA TTC AGC CCC CGT TAC CT	Regulator				

Real-time PCR: Quantitative real-time PCR assay was conducted on the pig tissue samples as described by (Probert et al., 2004). The 25 μl PCR reaction included 1x TaqMan® Universal PCR Master Mix with UNG (Applied Biosystems, France), 0.3 μM of each primer (F:GCTCGGTTGCCAATATCAATGC and R:GGGTAAAGCGTCGCCAGAAG) and 0.1 μM , TaqMan® *IS711* probe (5'FAM-AAATCTTCCACCTTGCCCTTGCCATCA-3' TAMRA) (MWG Biotech, Germany) with 2 μl of DNA template. The PCR condition consisted of initially

incubated cycle of 10 minutes at 95°C, followed by 45 cycles of denaturation at 95°C for 15seconds, annealing and extension at 60°C for 1 minute. The PCR reaction was performed on Step One plus PCR machine (Applied Biosystems, France). Probert et al. (2004) criteria were used for interpretation: A sample with a fluorescence signal 30 times greater than the mean standard deviation in all wells over cycles 2 through 10 was considered a positive result, whereas a sample yielding a fluorescence signal less than this threshold value was considered a negative result.

6.3.9 Ethical approval

Ethics approval for the study was obtained from ARC-OVI (Agricultural Research Council – Onderstepoort Veterinary Institute) Animals Ethics Committee (AEC12-16), University of Pretoria Animal Use and Care Committee (V089-16). Section 20 approval was granted according to Act 35 of 1984 by the Directorate of Animal Health, South Africa.

6.4 Results

In this study, 142 animals were samples and distributed as 40.1% (57/142) from sheep and 59.8% from (85/142) pigs from the eight abattoirs.

6.4.1 Sheep population results

Of the 57 slaughtered sheep sampled, 56.1% (32/57) were female and 43.9% (25/57) were male. Classification of the sheep by age showed 98.2% (56/57) as adults and 1.8% (1/57) as young. In accordance with the abattoir type sampled, 52.6% (30/57) sheep were from HT abattoirs, while 47.4% (27/57) were from LT abattoirs. All the sheep were of the Dorper breed.

For sheep, serological results for *B. ovis* antibodies detection with iELISA was 1.80% (1/57, 95%CI=0.04-9.39). All the 57 samples were negative (0.0%) for *B. melitensis* on RBT and iELISA. The sheep tissue samples were 93.0% (53/57) ITS-PCR positive while the isolation rate from the bacteriological method (culture and isolation) was 59.6% (34/57). Of the 34 *Brucella* suspect isolates, 18 were characterized as *B. melitensis* and seven *B. ovis* (total 25) using the AMOS-PCR assay. The Bruce-Ladder PCR assay did not detect any of the isolates as the *B. melitensis* Rev1 vaccine strain.

The association between the sex ($P=1.00$) and age ($P=1.00$) with the detection of *Brucella* DNA by PCR from the sheep tissue samples were statistically insignificant, but the association of the abattoir types ($P=0.00$) with a detection rate of *Brucella* DNA by PCR from the sheep tissue samples was statistically significant (Table 6.3). The measure of association between

the sex ($P=0.44$), age ($P=0.84$), abattoir types ($P=0.26$) with the isolation rate by culture were statistically insignificant (Table 6.4).

Table 6.3: *Brucella* DNA detection rate in sheep by molecular method stratified by sex, age and type of abattoirs sampled.

Animal demography	Total	PCR (%)	OR (95% CI)	Chi (X) ²	P-value
Sex					
Female	32	30 (93.8)	1.30 (0.17-9.97)	0.00	1.00
Male	25	23 (92.0)			
Age					
Adult	56	52 (92.9)	undefined	0.00	1.00
Young	1	0 (0.00)			
Abattoir type					
High throughput	30	30 (100.0)	undefined	39.4	0.00
Low throughput	27	4 (14.8)			

%- percentage, OR-odds ratio

Table 6.4: Isolation rate of *Brucella* spp. in sheep by bacteriological method stratified by sex, age and type of abattoirs sampled.

Animal demography	Total	Isolates (%)	OR (95% CI)	Chi (X) ²	P-value
Sex					
Female	32	21 (65.6)	1.76 (0.06-5.14)	0.59	0.44
Male	25	13 (52.0)			
Age					
Adult	56	34 (60.7)	undefined	0.04	0.84
Young	1	0 (0.00)			
Abattoir type					
High throughput	30	23 (76.7)	2.26 (0.72-7.08)	1.27	0.26
Low throughput	27	16 (59.2)			

%- percentage, OR-odds ratio

6.4.2 Pig population results

Eighty-five pigs were sampled of which 31.8% (27/85) were females and 68.2% (58/85) were males. Of these pigs, 62.4% (53/85) were adults and 37.6% (32/85) were young. According to abattoirs sampled, 64.7% (55/85) were from the HT abattoirs, while 35.3% (30/85) were from the LT abattoirs. All the pigs were of the large white breed. The pig tissue samples were 27.1% (23/85) ITS-PCR positive. However the qPCR using the genus specific on the pig samples had low CT values (36-40) and was negative according to the criteria of Probert et al. (2004) (Figure 6.1) and none amplified using AMOS-PCR. None (0.0%) of the pigs was positive on serology or on culture and isolation.

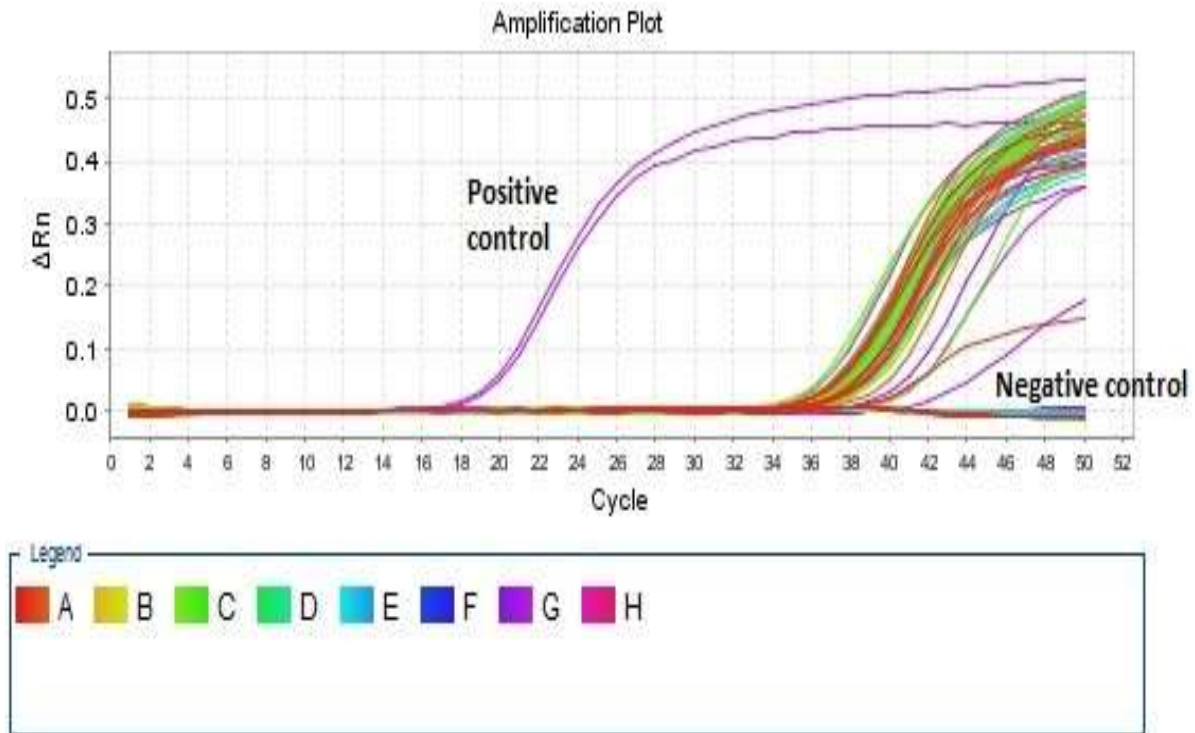


Figure 6-1: Fluorescence of the *Brucella* genus specific *bcs*p 31 probe in quantitative real time PCR assay using tissue samples of pigs. *B. abortus* bv. 1 strain REF 544 and sterile water were used as the positive and negative controls. The CT values for pig tissues from the abattoirs were between 36-40 and therefore the PCR results is interpreted as negative according to Probert et al. (2004) criteria.

The measure of association between the age ($P=0.00$) and abattoir type ($P=0.00$) with the *Brucella* DNA detection rate by ITS-PCR from the pig tissues was statistically significant, while the measure of association between the sex ($P=0.92$) with the *Brucella* DNA detection rate by PCR was not statistically significant (Table 6.5).

Table 6.5: *Brucella* DNA detection rate in pigs by molecular method stratified by sex, age and type of abattoirs sampled.

Animal demography	Total	ITS-PCR (%)	OR (95% CI)	Chi (X) ²	P-value
Sex					
Female	27	8 (29.6)	1.20 (0.44-3.33)	0.01	0.92
Male	58	15 (25.9)			
Age					
Adult	53	5 (9.43)	0.08 (0.02-0.26)	19.8	0.00
Young	32	18 (56.2)			
Abattoir type					
High throughput	55	4 (7.27)	0.05 (0.01-0.16)	28.1	0.00
Low throughput	30	19 (63.3)			

6.4.3 Frequency of isolation of *Brucella* organism from the tissues (lymph nodes, spleen and liver) of sampled slaughter sheep

6.4.3.1 Sheep population

From the total 53 PCR-positive sheep, the frequency of isolation of *Brucella* spp. was 64.2% (34/53, 95%CI=49.2-76.9). The isolation rate from all three tissue samples among the 34 sheep is summarized in Table 6.6.

Of these 34 sheep with positive *Brucella* isolates, the frequency of isolation by sex was 61.8% (21/34, 95%CI=43.6-77.8) in the females and 38.2% (12/34, 95%CI=22.2-56.4) in the males; and by age was 100.0% (34/34, 95%CI=89.7-100) in adults and 0.0% (0/34, 95%CI=0.00-10.3) in the young sheep. For abattoir types, the frequency of isolation was 67.6% (23/34, 95%CI=49.5-82.6) and 32.4% (11/34, 95%CI=17.4-50.5) in the HT and LT abattoirs respectively.

The isolation rate of *Brucella* spp. from the lymph nodes, spleen and liver of the sheep was statistically significant (P=0.03).

Table 6.6: Frequency of isolation of *Brucella* spp. from tissue samples of slaughtered sheep from abattoirs in Gauteng province, South Africa.

Animal species	No. of animals (No. with isolates)	% Positive (95%CI)	Lymph node (%)*	Spleen (%)*	Liver (%)*	p-value
Sheep	57 (34)	59.7 (45.8-72.4)	18 (53.0)	11 (32.4)	24 (70.6)	0.03

*Of the culture positive samples

6.5 Discussion

This study has shown that the prevalence of brucellosis among the slaughter sheep at the Gauteng abattoir is 59.7% using the gold standard of isolation method and identified using AMOS-PCR. *Brucella melitensis* isolates were isolated from 31.6% (18/57) sheep tissue although the sheep were all seronegative and *B. ovnis* isolates were isolated from 12.3% (7/57) of sheep and of which one was iELISA seropositive. Brucellosis could not be detected in the pig samples using serology, culture and PCR. The ITS-PCR detected *Brucella* DNA in the tissues, but this PCR is not an OIE recommended and validated PCR. Furthermore, the ITS-PCR results could not be confirmed using qPCR and AMOS-PCR. The Brucellosis control scheme in South Africa is biased towards cattle. Porcine brucellosis is not tested as *B. suis* does not occur in South Africa and testing for sheep and goat brucellosis caused by *B. melitensis* is not routinely screened and only become compulsory for high-risk herds or during

outbreaks. *Brucella melitensis* has been detected in sheep and goats in South Africa in 1965 (Van Drimmelen, 1965), 1989 (Ribeiro et al., 1990) and 1994 (Reichel et al., 1996), but were controlled through test and slaughter. *Brucella melitensis* have been isolated in 2015 from a human in South Africa, which should raise concerns as human brucellosis indicate presence in animals. Due to the predominant voluntary nature of the brucellosis testing scheme in South Africa, abattoir survey conducted in this study provided useful baseline data on the seroprevalence of brucellosis in the sheep and pig population within the Gauteng region.

Retrospective analysis of studies in southern Africa using serological assays alone indicated a low prevalence of brucellosis in sheep and goats whereas *Brucella* detected with culture and PCR assay indicated a much higher prevalence. As mentioned, Simpson et al. (2018) reported a 0.17% seroprevalence of caprine brucellosis in South Africa, while Madzingira and McCrindle (2015) reported a 0.14% seroprevalence of ovine brucellosis and 0.00% seroprevalence of *B. melitensis* and *B. ovis* from sheep samples in Namibia using only serum samples. However, in the Eastern Cape Province of South Africa, *B. melitensis* was isolated from 2.9% sheep and 6.3% goats using blood, milk and lymph nodes samples and identified using AMOS PCR (Caine et al., 2017). This underscores the fact that direct detection of the *Brucellae* from slaughter animals' tissues can provides a sensitive diagnostic approach for brucellosis on either seropositive or seronegative animals. The diagnostic strategy used in our study demonstrated that serological assay alone will not detect all brucellosis-infected animals, as most animals may be serologically negative but the *Brucella* organism may persist in the tissues, especially in chronically or latently infected animals (Olsen and Palmer, 2014, Radostits et al., 2006). The strategic application of bacteriology, serology and PCR was shown to result in increased detection of brucellosis in the animals.

All the 57 sheep tested in this study were seronegative for *B. melitensis* using RBT and iELISA, but 1.8% (95%CI=0.04-9.39) was seropositive for *B. ovis* on iELISA. A seroprevalence rate of 3.03% for *B. melitensis* has been reported in KwaZulu Natal province of South Africa using a combination of serological tests which included RBT, CFT and SAT for *B. melitensis* during an outbreak (Emslie and Nel, 2002). Unlike the 1.8% seroprevalence rate of *B. ovis* in our study, a 5.0% seroprevalence rate of *B. ovis* was reported in sheep in South Africa, using the CFT alone (Maria et al., 1972). This underscores the importance of using different serological tests in making a diagnosis.

It is known that no serological test is 100% accurate and therefore it requires the use of two tests to predict brucellosis. The probability of false-negative test result is high when only one test is used compared to when several serological tests are used. In our study, most of the titres (98%-110%) in the sera tested were just below the cut-off point recommended by the

commercial IDEXX Brucellosis Antibody Test iELISA kit (France). This suggests that, the cut-off point for each region should be determined according to the prevalence rate of brucellosis in the region, and is a further indication that antigens used in one region may not be applicable (sensitivity and specificity) with other regions. Another known problem is that the antigens used in most serological tests may vary from one region or country to another and, may vary between laboratories (Blasco, 1990, Bulgin, 1990, West and Bruce, 1991). This may have affected the results in our study. Another plausible factor is that the *Brucellae* may have localized in the tissues of these sheep and serological tests were unable to demonstrate antibodies against *Brucella* spp. in sheep (Alton, 1990b, Bulgin, 1990, Olsen and Palmer, 2014, Radostits et al., 2006). Another possibility is that seronegative sheep in this study may have been sent to the abattoir from the farms or owners knowing the serology status of these animals, but *Brucella* was detected in the tissues of the sheep with PCR and isolation. This is a plausible reason for some farmers to try to circumvent the detection of positive animals and to have their sheep passed for slaughtering at the unsuspecting abattoirs. Another possibility is it has also been observed that immune-compromised animals may not develop antibodies against antigens in serological tests. It has therefore been recommended that molecular assays, such as the PCR, which is sensitive and specific, be used to demonstrate the presence of *Brucella* DNA from the tissues (Garin-Bastuji et al., 1998, Keid et al., 2007). Further evaluation of these practices should be conducted and following seronegative sheep to the abattoirs to critically evaluate this possibility.

An investigation on different rose Bengal antigens indicated the reason for inconsistent RBT results as the lack of international standardization of rose Bengal antigen that result in differences in the pH and cell concentration agglutination with the international standard anti-*B. abortus* serum (Blasco et al., 1994). The authors also reported that in the sheep and goats population tested, RBT was not sensitive on serum samples from culture positive sheep and goats and CFT was even less sensitive than the RBT test (Blasco et al., 1994). There is no general consensus on which *Brucella* spp. and biovar should be used in making a diagnosis using the Rose Bengal antigen for *B. melitensis*, therefore the reliability on this test still remains unresolved (Blasco et al., 1994). Culture of A-dominant antigen of *B. abortus* bv 1 strain is usually used (Alton et al., 1988), and infection from M-dominant antigen of *B. melitensis* bv 1 may be misdiagnosed (Alton, 1990b, MacMillan, 1990). This could explain our results, where no seropositive sheep was detected using RBT and iELISA.

It is therefore significant that in this study it was detected that though all the sheep tested were seronegative (RBT and iELISA) for *B. melitensis* and one (1.8%) sheep was seropositive for *B. ovis*, 93.0% (53/57) of the sheep were positive on ITS-PCR assay conducted on the tissue samples (lymph nodes, spleen and liver) and 44.0% (25/57) were AMOS-PCR positive. This

is not surprising as detection of *Brucella* through molecular methods in seronegative animals have also been demonstrated in cattle in other studies (Islam et al., 2018, Sabrina et al., 2018). Furthermore, the isolation rate for *Brucella* spp. was 64.1% (34/53) in the ITS-PCR positive sheep and 47.1% (25/53) in the AMOS-PCR positive sheep, an indication that these sheep had *Brucella* spp. in their tissues with the potential to shed the pathogen. This suggests that the sheep may have been chronically infected, and diagnosis with serology alone would not have indicated positivity, hence, the spread of infection by the sheep would have continued unabated within the flock. The importance of this finding has demonstrated the advantage of using PCR as an additional diagnostic tool for brucellosis in sheep. Many studies have highlighted the importance of PCR as more reliable diagnostic tool. The detection of *B. abortus* and *B. melitensis* in blood and tissues from cattle, sheep, goats and camels using PCR have already been documented (Khamesipour et al., 2013, Khamesipour et al., 2014). Furthermore, the use of PCR in another study on aborted fetuses from a sheep population has reported sensitivity and specificity of 97.4% and 100% respectively (Leyla et al., 2003). In another study on slaughtered sheep in Turkey, diagnosis using PCR detected a higher brucellosis rate of 29.0% against 27.7% using serology on blood and lymphoid tissues respectively. In this same study, a 17.2% isolation rate was reported for the PCR positives lymphoid tissues of the slaughtered sheep (Ilhan et al., 2008). An important similarity in these studies and our abattoir study is the detection of *Brucella* DNA in the lymph nodes of seronegative and seropositive sheep. The detection rate of 29.0% in the study in Turkey using PCR and the isolation rate of 17.2% from PCR-positive tissues is lower than the 93.0% ITS-PCR, 44.0% AMOS PCR and 59.6% isolation of *Brucella* spp. found in the slaughter sheep population in this study in the Gauteng abattoirs. Furthermore, there are reports of seronegative animals testing positive on PCR. Junqueira Junior et al. (2013) reported out of 88 seronegative bulls, 30.7% were positive for brucellosis on PCR. In another study in a seronegative goat population previously vaccinated with *B. abortus* RB51 vaccine, *B. melitensis* was isolated from one animal (Herrera et al., 2011). Arellano-Reynoso et al. (2013) also reported the isolation of *B. abortus* from two of 209 seronegative cow that were vaccinated with RB 51 strain. PCR detected *Brucella* DNA in 84.8% (759/895) of the examined samples of camels in Sudan, of which 15.5% (118/759) were serologically negative (Gwida et al., 2011). Serum samples collected very recently after abortion from four buffaloes and six goats gave negative results on serology, but after three weeks post abortion, a strong positive reactions on RBT, CFT and ELISA were produced from these seronegative animals (Wareth et al., 2015). These findings show that the use of direct PCR methods on field sample should be encouraged as its performance has been found to supersede serological methods of diagnosis (Bricker, 2002, OIE, 2009). It cannot be over-emphasized that the findings in these studies underscore the fact that seronegative sheep can

test positive with the use of a more sensitive method, such as the PCR assay, when making a diagnosis of brucellosis in the sheep population.

However due to a bias in the sample size of sheep in this study, a thorough investigation with larger sample size and known seropositive and negative animals, if possible, is necessary to investigate brucellosis in sheep in South Africa. Contamination of the sheep tissues may have been possible during slaughtering process at the abattoirs. It is obvious to anyone familiar with the methods of dressing and cutting meat that the presence of viable organisms in one carcass poses a very real threat of contamination to subsequently processed carcasses through the media of knives, saws, holstering lines, cleavers, grinders, chopping blocks, and butcher's hands or gloves (Sadler, 1960). The contamination of meat at the abattoir is a critical factor to be considered in the evaluated of the role of meat in the epidemiology of brucellosis (Sadler, 1960). Contamination at the abattoirs or laboratory cannot be confirmed in our study but importantly, this underscores the fact that clean carcasses or tissue samples can be contaminated by infected carcasses or tissues if proper precautionary measures are not taken. Contaminated carcasses can still be a source of infection to susceptible abattoir workers, laboratory personnel and those who process meat far away from the abattoirs. In the slaughtered sheep population, a higher isolation rate for *Brucella* was observed in the liver (70.6%) compared to the other organs and this was statistically significant ($P=0.03$). This may suggest a chronic infection in the sheep population (Akritidis et al., 2007). This result in part may explain why antibodies were not detected in the sera. However, this is significant because the application of PCR to detect *Brucella* DNA in animal tissues can be used to diagnose brucellosis in immune-compromised animals that are unable to seroconvert following exposure and infection, and in animals where the *Brucella* organism, which is an intra-cellular organism, is localized in the tissues, such that serology may not be able to diagnose the infection from the serum samples (Radostits et al., 2006). Of the sheep tested, the frequency of isolation was significantly ($P=0.03$) higher for the liver samples (42.1%) compared to the lymph nodes (31.6%) and spleen (19.3%) samples. Therefore, sampling of sheep liver for the detection of *Brucella* spp. may be the preferred option for isolating the pathogen. More studies are advocated to verify these results.

In this study, all the pigs samples tested were seronegative for antibodies against *B. suis*, a finding in agreement with the observation that the identification of the pathogen in pigs is difficult (Alton, 1990a, Radostits et al., 2006), and diagnosis using serology alone may be doubtful to ascertain the status of brucellosis in pigs or wild boars (Hinić et al., 2009). Erume et al. (2016) further highlighted the difficulties associated with the diagnosis of brucellosis in pigs with the report that a study on pig population in Uganda revealed that serological assays did not perform well. The authors further used molecular method for the detection of *Brucella*

DNA from the serum samples from the pigs and reported that PCR did not detect *Brucella* DNA in the extracted DNA from the pigs' sera (Erume et al., 2016). It has been suggested that pig serum samples may not be the best type of sample for molecular examination, because the organism can only be found in the serum of pigs that were bacteremic at the time of sampling, and more importantly, brucellosis in pigs is a chronic disease (Godfroid et al., 2013, Hutchings, 1950). The *Brucella* organism is usually widely spread in the pigs body, unlike what is obtainable in ruminants (Radostits et al., 2006). Brucellosis seroprevalence studies in pigs recorded 0.0% rates in Nigeria (Cadmus et al., 2006) and Zambia (Stafford et al., 1992) which were inconclusive because the use of only serological test is unsatisfactory in pigs (Corbel, 2006, Radostits et al., 2006). However, in this study, in addition to serological assays, molecular assay, specifically the ITS-PCR used was able to detect *Brucella*-like DNA in 29.4% (25/85) of the slaughtered pigs which were all seronegative.

There have been the detection of *Brucella* DNA in the tissues of seronegative wild pigs in Switzerland (Hinić et al., 2009). However, as *B. suis* has not been confirmed in South Africa, the suspect ITS-PCR samples were tested with a more sensitive qPCR (OIE recommended) using a *Brucella* genus specific probe targeting the *bcsp31* genes. As mentioned, the ITS-PCR is not an OIE recommended PCR and not validated in South Africa. The ITS-PCR results could not be confirmed with OIE recommended PCR assays and therefore the pig samples are negative for brucellosis.

Both the ITS-PCR and qPCR have been used to test closely-related bacterial species and found to be specific (Keid et al., 2007, Probert et al., 2004), but these PCRs have to be validated in South Africa to ensure that cross-reaction with any other closely related bacteria occurring in South Africa does not occur. Validation of the ITS-PCR and other genus specific PCR methods is important, as potential novel *Brucella* spp. from frogs were reported that were phenotypically identified as *Ochrobactrum anthropi* but genetically related to *Brucella* (Eisenberg et al., 2012). There is a possibility that the *Brucella* DNA detected in the pig samples using ITS-PCR might not be *Brucella* and/or *B. suis*. Furthermore, this study expresses caution in the interpretation of ITS-PCR data as other factors may have been responsible for this result as such more research should be conducted to ascertain the findings.

6.6 Limitations of the study

The limitations of this study are the use of the ITS-PCR that needs validation in South Africa. The cut-off for the ELISA also needs to be validated in South Africa. Good sampling size was not achievable; as such it will be impossible to know the epidemiological situation in South

Africa. A follow up visit to the farm sources was not conducted because of lack of cooperation from the state veterinarians.

6.7 Recommendations

This study recommends review or revised testing methods for the sheep and pigs. A validation of molecular tests should be conducted to ascertain the validity of results obtainable from the pig population. Abattoir studies can be replicated in other provinces of the country to obtain a better surveillance results for both sheep and pig population in the country.

6.8 Conclusions

This study has confirmed that as a diagnostic strategy, more than one diagnostic method or test should be instituted for the diagnosis of brucellosis in animals, especially with reference to the results derived from testing tissues (isolation and PCR) of animals slaughtered at abattoirs in the Gauteng province or the country at large. A variety of samples could be collected at slaughter which may be difficult if live animals are to be sampled on farms at several locations. This study has not been able to verify the occurrence of brucellosis in the pig population in South Africa. Reliance on the methods used on the pigs may not be satisfactory.

6.9 References

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Chapter 7

7.1 General discussion and conclusions

There are limited recent empirical data or published reports on the existence or prevalence of brucellosis in the livestock industry in South Africa. Currently in South Africa, the databases on brucellosis are fragmented in several laboratories testing for brucellosis and therefore difficult to access any organized data to assess the status of the disease. As mentioned, the testing scheme for bovine brucellosis is compulsory for only high-risk herds that have been confirmed or suspected of infection using RBT and CFT tests. Entering the brucellosis testing scheme is voluntary for all other bovine herds and livestock owners. Data extracted from the retrospective studies can therefore not be assumed to depict the epidemiology of brucellosis in the country. Therefore, caution must be taken with the numbers calculated in this study, given that there might be an important bias in the selection of animal sampled for brucellosis serology and may not reflect reality in the country. However, it is recommended from this study and from suggestions from DAFF that there is a need for correct and compulsory centralized reporting of essential information from all laboratories testing for brucellosis.

In the context of the problems established from the retrospective data of which only serological tests were conducted, coupled with existing flaws in recording systems, and major inferences that cannot be made because important variables were missing in the data, such as the age, vaccination status, sex, breed and sources of the animals, a study to evaluate these problems is important. It was therefore pertinent to investigate the current infection or disease status of this zoonotic disease of brucellosis in a provincial abattoir-based study using serology, molecular and bacteriological methods. This investigation will establish its occurrence in the livestock, which have the potential to pose food safety concerns to the population using serum and tissue samples from the animals. Although the abattoirs cannot be used to establish the prevalence of brucellosis in the livestock population in the country, this study generated important and relevant data for the province as well as a template for province and/ or country surveillance. This study emphasizes the lack of information and research regarding brucellosis. Proper mapping of the population at risk and the distribution of the diseases will ensure, the efficient administration of resources to protect the health of the animal and human populations in affected areas (WHO, 2007).

The abattoir study enabled a polyphasic approach (serology, culturing and PCR) as serum and tissue samples from livestock were available. Most significantly, the isolation of *Brucella*

spp. in the abattoir study has revealed the occurrence of *B. melitensis* bv 2 and 3 in the cattle population in the country for the first time. In South Africa, testing scheme for bovine brucellosis (established under section 10 of the Animal Disease act 35) is compulsory for only high-risk herds that have been confirmed, or suspected of infection. This is novel and makes a significant contribution to science. The occurrence of *B. melitensis* in the cattle population indicate spill over to cattle from other animals species. The polyphasic approach with its broader possibility of diagnosis is only possible using abattoir samples. It is recommended therefore that abattoir facilities are included in brucellosis scheme for surveillance to enable identification of *Brucella* to species and biovar level in addition to making serological testing and surveillance for cattle, sheep and goats compulsory.

The genus specific ITS-PCR assay, which can be used as a screening test, needs to be validated in South Africa. The ITS-PCR detected the frequency of *Brucella* DNA among the sampled cattle population to be 12.5%. The bacteriological method known for its low sensitivity for the isolation of *Brucella* spp. yielded an isolation rate of 5.5% in the cattle population and this was indicative of the seroprevalence (using RBT and iELISA) from this study. Serology indicated a 5.5% seroprevalence for cattle in the abattoirs study, which is comparable to 5.23% seroprevalence recorded in the retrospective studies. In the sheep a 2.09% seroprevalence in the retrospective data was observed, while serology was seronegative in the sheep from the abattoir samples. For the pigs in the retrospective study, OIE recommendation to differentiate *Brucella* infection from *Yersinia enterocolitica* O:9 was not conducted. However, brucellosis could not be detected in the pig samples from the abattoirs using serology, culture and PCR. The ITS-PCR detected *Brucella* DNA in the tissue, but this PCR is not an OIE recommended and validated PCR. Furthermore, the ITS-PCR results could not be confirmed using qPCR and AMOS-PCR and therefore the pig samples were concluded to be negative for brucellosis or until otherwise proven.

Brucellosis is a zoonotic disease, which is an area of concern for One Health (Franc et al., 2018, Godfroid et al., 2014). To date, there are no empirical data or published reports on the risk factors, occurrence or prevalence of brucellosis among the abattoir workers in South Africa. It was therefore important to conduct a provincial, abattoir-based study on the risk factors that predispose abattoir workers to zoonotic infection. Importantly, brucellosis has been detected from animal samples from the abattoirs. This emphasizes the potential of spill over or exposure of *Brucella* to the abattoir workers. Data from this study have been used to assess the knowledge among abattoir managers and staff on zoonoses as occupational hazard at the abattoir facilities, and to assess the correlation between how the abattoir workers perform their duties at the abattoirs, and the standards the managers have instituted for the protection and the public health safety of the abattoir workers. In the context of the abattoir

workers, the study assessed the possibility of interruption of zoonotic disease transmission cycle. Risk factors that could predispose these workers to infection were assessed, and this was because the transmission of diseases requires a source of infection (animals), a susceptible host (abattoir employee), a route of transmission (contact -direct or indirect-, aerosol or vector borne), and a portal of entry, such as an open wound or mucous membrane (Scheftel et al., 2010). In our study, 84.0% of our human respondents confirmed to have experienced hand cut injuries at least once while performing their duties at the abattoirs. These cut injuries are portals of entry for infectious organisms when in direct or indirect contact with infected animal's blood or fluid. A proportion of 70.0%, 58.0% and 90.2% of our respondents did not use facial masks, hand gloves and goggles respectively. All these risk factors measured gave an evidence-based data to proceed in testing the abattoir workers for brucellosis. Therefore, the investigation on the seroprevalence of brucellosis among abattoir workers in Gauteng province in South Africa was informed by the baseline data generated from the initial study on risk factors. As such, an overall seroprevalence rate of brucellosis using combined serological tests (RBT, BrucellaCap, IgG ELISA) among abattoir workers in the Gauteng province of South Africa was determined to be 20.4%. The risk factors found to be associated with seropositivity were sex, job description, days at work and working on other farms apart from the abattoir. This is the first abattoir study on human brucellosis to be conducted among abattoir workers in South Africa. It provides relevant data on the group of workers and further reiterates the need to replicate the study in other provinces, to fully understand the status of the infection in the occupationally exposed stakeholders in the country.

7.2 General conclusions

In conclusion, brucellosis is enzootic in South Africa and our observation has shown this to be true from samples tested from the Gauteng province abattoirs. Information about the prevalence of the disease in low and middle income countries is very scarce and tends to hinder the control of the disease, this study has provided an understanding of how the occurrence of the brucellosis is distributed within Gauteng province and the country, at large. The information from this study can be used pro-actively to drive, monitor, change or formulate policies to mitigate the challenges brought about by brucellosis in the livestock sector of the country. With the increased knowledge now available globally, it is not acceptable for the knowledge gap detected in the current study to be left unaddressed. Our study has provided evidence-based data, that *B. melitensis* may be circulating in the cattle population in South Africa. This suggests a potential risk to consumers of unpasteurized milk or milk products and consumers of uncooked or undercooked meat or meat products. In addition, infected animals

pose a risk of occupational zoonotic infection to abattoir workers and workers on farms from where the slaughter animals originated. A multidisciplinary One Health approach has been successfully applied in this study and the interaction between animals and humans in the abattoir environment has been evaluated to be a plausible source of brucellosis to the workers. It is strongly suggested to identify acute brucellosis in abattoir worker to clearly identify the transmission route (Skin, Aerosol, and Hygiene) and to identify the *Brucella* species to be able to trace the infection to its animal reservoirs in South Africa.

7.3 References

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Appendix 1

VERBAL QUESTIONNAIRE

This questionnaire has four (4) sections as shown below;

1. Background information about the interviewer
 - Language
 - name of interviewer
 - Date of interview
 - Date of entry into the computer
2. Background information about respondent
 - Respondents Work address
 - Name
 - Age
 - Marital status
 - Sex
 - Years of work at abattoir
 - Job description
 - Section of animal slaughter
3. closed ended questions
 - Specific questions on practices in the abattoir
 - Questions on protective clothing
 - Passed history of illnesses
4. Information about passed infections

Section 1: Background information about the interview

2.1 Interview language (Zulu,Pedi, Suthu, Xitsonga, English, Afrikaans) _____

2.2 Interviewer name _____

	Day/month/year
Date of first interview attempt	
Date and time arranged for second interview attempt	
Date and time arranged for third interview attempt	
Date of interview	
Date form checked by supervisor	
Date entered in computer	

Section 2: Background information of the individual

(to be filled in before interview)

2.1 Address of the Abattoir / GPS

2.2 Name of respondent (will be kept confidential)

2.3 Sex of respondent 1. Male 2. Female 3. Unknown

2.2 Age of respondent 15-30

31-60

2.3 Marital status 1. Married 2. Single 3. Divorced

2.4 Job description

Butcher

Inspector

Transporter

Others _____

2.5 How long have you worked in the abattoir?

One year

Two years

More than three years

2.6 What animal section do you work in?

Cattle

Sheep/goat

Pig

Games

All sections

Section 3: Information about practices by respondent

3.1 Do you eat raw meat? 1. Yes 2. No

(If yes: how often do you eat raw meat? if no: go to question 3.2)

Regularly

Sometimes

3.2 Have you ever cut your hands during work 1. Yes 2. No

3.3 Do you work when you have wound or injury? 1. Yes 2. No

3.4 Do you sometimes have animal secretions splash on your face

1. Yes 2. No

3.5 Do you wash your hand regularly during work 1. Yes 2. No

(If yes: How often?; in no go to question 3.6)

Once

Twice

Every time

3.6 Do you wear mask during work? 1. Yes 2. No

(If yes: How often?: if no go to question 3.7)

Sometimes

Always

3.7 Do you wear gloves during work? 1. Yes 2. No

(If yes: How often?: if no go to question 3.8)

Sometimes

Always

3.8 Do you wear over all clothing during work? 1. Yes 2. No

(If yes: How often?: if no go to question 3.9)

Sometimes

Always

3.9 Do you wear boots during work? 1. Yes 2. No

(If yes: How often?: if no go to question 4.0)

Sometimes

Always

4.0 Do you wear protective glasses during work? 1. Yes 2. No

(If yes: How often?: if no go to Section 4)

Sometimes

Always

Section 4: Information about passed infections

4.1 Have you ever been sick from the abattoir?

(If yes: go to question 4.1.1 if no go to question 4.2)

4.1.1 What kind of illness? : _____

4.2 Have you ever been diagnosed with illness from the abattoir?

(If yes: go to question 4.2.1 if no go to question 4.3)

4.2.1 What was the diagnosis?

Brucellosis

Leptospirosis

Tuberculosis

Cystercercosis

Q-Fever

Don't know

Others _____

4.3 Do you think you can get sick from the abattoir? 1. Yes 2. No

Appendix 2

Section 1: Background information of the individual

1.1 Address of the Abattoir/GPS

1.2 Name of respondent (will be kept confidential)

1.3 Sex of respondent 1. Male 2. Female

1.4 Age of respondent

1.5 Marital status 1. Married 2. Single

1.6 Job description

Butcher/slaughter man

Inspector

Transporter

Others _____

1.7 How long have you worked in the abattoir?

One year

Two years

Three years and above

1.8 How many days do you work in the abattoir per week?

Everyday

5-7 days

3—4

1---2

1.9 What animal section do you work in?

Cattle

Sheep/goat

Pig

Games

All sections

1.10 Do you take care of animals at home or work on farms? 1. Yes 2. No

If yes, Indicate the types of animals you take care of: _____

- 1.11 Do you consume unpasteurized milk? 1. Yes 2. No
- 1.12 Do you consume uncooked or undercooked meat? 1. Yes 2. No
- 1.13 Do you slaughter animals at home? 1. Yes 2. No
- 1.14 Do you have personal protective gears? 1. Yes 2. No

(If yes: go to question 3.13.1 if no go to question 3.14)

- 1.15 Do you wear your personal protective gears? 1. Yes 2. No
- 1.16 Have you ever had any of the symptoms below in the abattoir?
1. Yes 2. No

- | | | | | | |
|------------------|--------------------------|-------------|--------------------------|---------------------|--------------------------|
| Fever | <input type="checkbox"/> | Coughing | <input type="checkbox"/> | Nausea and vomiting | <input type="checkbox"/> |
| Cold | <input type="checkbox"/> | Body pain | <input type="checkbox"/> | | |
| Loss of appetite | <input type="checkbox"/> | Night sweat | <input type="checkbox"/> | | |
| Seizures | <input type="checkbox"/> | Diarrhoea | <input type="checkbox"/> | | |
| Back pain | <input type="checkbox"/> | Weakness | <input type="checkbox"/> | | |

- 1.17 Where do you get your drinking water at the abattoir?

- Taps
- Dam/well
- Other

- 1.18 Where do you get your drinking water at home?

- Taps
- Dam/well
- Other

- 1.19 If not from the tap, how do you treat it?

- Bleach
- Boiling
- Other

SECTION 2 :- ZOOSES

Brucellosis

- 2.1 Do you know what brucellosis disease is? 1. Yes 2. No

(If yes: go to question 2.2 if no go to question 2.3)

- 2.2 Do you think you can contract brucellosis from animals abattoir
1. Yes 2. No
- 2.3 Have you ever had hand injuries working at the abattoir? 1. Yes 2. No
- 2.4 Within the last 12 months, have you been diagnosed of having brucellosis?
1. Yes 2. No

If 'yes', indicate the clinical symptoms experienced: _____

Leptospirosis

- 2.5 Do you know what leptospirosis disease is? 1. Yes 2. No
- (If yes: go to question 2.6 if no go to question 2.7)**
- 2.6 Do you think you can get leptospirosis from abattoir? 1. Yes 2. No
- 2.7 Do you see rats around the abattoir? 1. Yes 2. No
- 2.8 Do water/blood splash unto your face in the abattoir? 1. Yes 2. No
- 2.9 Do you use water bodies around the abattoir? 1. Yes 2. No
- 2.10. Do you see dogs around the abattoir? 1. Yes 2. No
- 2.11 If you are a meat inspector or involved in slaughtering, within the last 12 months have you observed any carcass and mucous membranes with jaundice (yellow carcass)?
1. Yes 2. No 3. NA
- 2.12 Do you have dog/pets at home? 1. Yes 2. No

Within the last 12 months, have you been diagnosed of having clinical leptospirosis:

1. Yes 2. No

If 'yes', indicate the clinical symptoms experienced: _____

Tuberculosis

- 2.13 Do you know what tuberculosis is? 1. Yes 2. No
- 2.14 Have you been vaccinated against TB? 1. Yes 2. No
- 2.15 Have you ever been sick from TB? 1. Yes 2. No

If 'yes' what were the clinical symptoms you experienced _____

- 2.16 Has any member of your family been sick from TB before? 1. Yes 2. No

If 'yes' what were the clinical symptoms experienced? _____

- 2.17 Do you think you can get TB from animals? 1. Yes 2. No
- 2.18 Do you think you can infect animals with TB? 1. Yes 2. No

Taeniasis (tapeworm infection)

- 2.19 Do you know what taeniasis is? 1. Yes 2. No
- 2.20 Do you consume meat from animals that might have grazed in pastures contaminated with human faeces? 1. Yes 2. No
- 2.21 Do you consume meat from animals that might have drunk water contaminated with human faeces? 1. Yes 2. No
- 2.22 Have you ever had abdominal cramps or lost weight? 1. Yes 2. No
- 2.23 If you are a meat inspector or involved in slaughtering, within the last 12 months did you observe any cysts in the carcasses? 1. Yes 2. No 3. NA

If yes, overall, how many carcasses were affected in that period? _____

Human Cysticercosis

- 2.24 Do you know what human cysticercosis is? 1. Yes 2. No
- (If yes: go to question 2.25 if no go to question 2.26)**
- 2.25 Have you ever been diagnosed with cyticercosis? 1. Yes 2. No
- 2.26 Do you thoroughly wash your hands with soap hands every time after using the bathroom? 1. Yes 2. No
- 2.27 Do you wash your fruits and vegetables every time before eating them? 1. Yes 2. No
- 2.28 Have you ever had seizures? 1. Yes 2. No
- 2.29 Within the last 12 months have you been diagnosed of having taeniasis or cysticercosis? 1. Yes 2. No

If 'yes', what were the clinical symptoms you experienced: _____

Toxoplasmosis

- 2.30 Do you know what toxoplasmosis is? 1. Yes 2. No
- (If yes: go to question 2.31 if no go to question 2.32)**
- 2.31 Do you think you can get toxoplasmosis from animals? 1. Yes 2. No
- 2.32 Do you see cats around the abattoir? 1. Yes 2. No
- 2.33 Do you keep cats as pets at home? 1. Yes 2. No
- 2.34 Within the last 12 months, have you been diagnosed of having toxoplasmosis? 1. Yes 2. No

If 'yes' what were the clinical symptoms you experienced? _____

Q-fever

2.35 Do you know what Q-fever is? 1. Yes 2. No

(If yes: go to other questions if no stop questions)

2.36 Do you think you can contract Q-fever from animals? 1. Yes 2. No

2.37 Do you think you can contract Q-fever from the abattoir? 1. Yes 2. No

2.38 Do you think you can infect animals with Q-fever? 1. Yes 2. No

2.39 Within the last 12 months, have you been diagnosed of having Q-fever?
1 Yes 2. No

If 'yes' what were the clinical symptoms you experienced? _____

Any Other Comments _____

THANK YOU SO MUCH FOR PARTICIPATING IN THE SURVEY.

Appendix 3

Abattoir Manager questionnaire

1.1 Interview language (Zulu, Pedi, Suthu, Xitsonga, English, Afrikaans)

1.2 Interviewer's name _____

	Day/month/year
Date of first interview attempt	
Date and time arranged for second interview attempt	
Date and time arranged for third interview attempt	
Date of interview	
Date form checked by supervisor	
Date entered in computer	

1.3 How many abattoir workers do you have? _____

1.4 Do you know that your workers can get sick from the abattoir?

(If yes: go to question 1.2.1 if no go to question 1.5)

1.4.1 What kind of sickness do you think they can contract?

Brucellosis

Leptospirosis

Tuberculosis

Cystercercosis

Q-Fever

Don't know

Others _____

1.5 Do you educate your workers about zoonotic infections from the abattoir?

1. Yes 2. No

(If yes: go to question 1.3.1 if no go to question 1.4)

1.3.1 How often

Once a year

Twice a year

More than twice a year

1.6 Do you provide protective gears for your workers 1. Yes 2. No

(If yes: go to question 1.7 if no go to question 1.8)

1.7 Do you enforce your workers to wear the protective gears

1. Yes 2. No

(If yes: go to question 1.7.1 if no go to question 1.8)

1.7.1 How often do you enforce wearing protective gears

Always

Sometimes

1.8 Does your workers engage in risk practices that could expose them to infections in the abattoir? 1. Yes 2. No

(If yes: go to question 1.8.1 if no go to question 1.9)

1.8.1 What risk practices do they engage in?

Eating raw meat

Refusal to wear protective gears

Refusal to practice hygienic measures like hand washing

1.9 Has any of your workers been diagnosed with any zoonotic/occupational infection?

1. Yes 2. No

(If yes: go to question 1.9.1 if no go to question 1.10)

1.9.1 What kind of zoonotic infection did they contract?

Brucellosis

Leptospirosis

Tuberculosis

Cystercercosis

Taeniosis

Q-Fever

Don't know

Others _____

1.10 Do you test your workers for possible zoonotic infection? 1. Yes 2. No

(If yes: go to question 1.8.1)


- 1.10.1 How often
- Once a year
- Twice a year
- More than twice a year

Appendix 4

Educational Materials



Animal ethics approval



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee


PROJECT TITLE	Prevalence and characterization of <i>Brucella</i> spp. in slaughter animals in Gauteng Province abattoirs: Food safety implications for meat consumers and zoonotic hazards posed to abattoir workers (Phase 1)	
PROJECT NUMBER	V089-16	
RESEARCHER/PRINCIPAL INVESTIGATOR	Dr. F B Kolo	

STUDENT NUMBER (where applicable)	UP_11233509	
DISSERTATION/THESIS SUBMITTED FOR	PhD	

ANIMAL SPECIES	Bovine, Ovine, Caprine, Game Porcine	
NUMBER OF SAMPLES	1000	
Approval period to use animals for research/testing purposes	August 2016 – August 2017	
SUPERVISOR	Prof. H van Heerden	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 29 August 2016
CHAIRMAN: UP Animal Ethics Committee	Signature 

Human ethics approval

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance:

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/02/2022.
- IRB 0000 2295 ICRG0001792 Approved dd 22/04/2014 and Expires 03/14/2020.



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

23/11/2017

**Approval Certificate
New Application**

Ethics Reference No: 519/2017

Title: Prevalence and characterization of selected pathogens in slaughter animals in Gauteng Province abattoirs: Food safety implications for meat consumers and zoonotic hazards posed to abattoir workers

Dear Dr Francis FB Kolo

The **New Application** as supported by documents specified in your cover letter dated 18/10/2017 for your research received on the 18/10/2017, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 22/11/2017.

Please note the following about your ethics approval:

- Ethics Approval is valid for 1 year
- Please remember to use your protocol number (519/2017) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:

- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

*** Kindly collect your original signed approval certificate from our offices, Faculty of Health Sciences, Research Ethics Committee, Tswelopele Building, Room 4.35 / 4.60.*

Dr R Sommers; MBChB, MMed (Int); MPharm, PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

☎ 012 356 3084 ✉ desheba.bhehari@up.ac.za / fhethics@up.ac.za 🌐 <http://www.up.ac.za/healthethics>
📍 Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4, Room 60, Gezina, Pretoria

GDARD and Section 20 approvals



GAUTENG PROVINCE
AGRICULTURE AND RURAL DEVELOPMENT
REPUBLIC OF SOUTH AFRICA

4th Floor, Old Trust Bank Building, 64 Odendaal Street, Germiston
Private Bag X1099, Germiston, 1400
Tel: 011 821 7700
Fax: 011 821 7759
Email: chris.marufu@gauteng.gov.za
Website: <http://www.gdard.gpp.gov.za>

Enquiries: Dr Chris Marufu
Date: 21 June 2016
Reference: Permission to collect abattoir specimens

To: Whom it may concern

Dear Sir/Madam

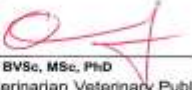
Re: Permission to collect abattoir specimens

This serves to acknowledge that I am aware of and have given permission for sample collection in the project "Prevalence and characterization of selected pathogens in slaughter animals at Gauteng Province abattoirs: Food safety implications for meat consumers and zoonotic risks posed to abattoir workers" that will be conducted in a collaborative project amongst GDARD, ARC-OVI and University of Pretoria, Department of Veterinary Diseases under the supervision of Abiodun Adesiyun and Henriette van Heerden. The project will involve the collection of serum, lymph nodes, granulomatous/tuberculous lesions, and kidneys from 15 multispecies abattoirs in Gauteng to determine the prevalence and characteristics (species/serotypes, antibiotic sensitivity, virulence/pathogenicity and genotypes) of selected pathogens (bacterial, protozoan and parasitic) in slaughter livestock and wildlife in the Province and to assess the potential health risk posed to abattoir workers.

I am aware that samples will be collected at abattoirs in the Gauteng province and will be processed at the ARC-OVI and BSL2 plus bacteriology laboratory at DVTD as allowed by section 20 approval. Samples collected from abattoirs will be transported to the laboratory under cover of the necessary red cross permits obtainable from the relevant State Veterinarian in Gauteng. Disposal of any sample materials and waste after intended use will be by incineration as detailed in the section 20 application.

Please direct any queries regarding the above to me.

Yours faithfully


Dr MC Marufu BVSc, MSc, PhD
Chief State Veterinarian Veterinary Public Health

DR MC MARUFU
BVSc MSc Reg: D07/9133
Chief State Veterinarian





agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Henry Goloko • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HenryG@daff.gov.za
Reference: 12/11/1/1/6

Dr Henriette van Heerden
Department of Veterinary Tropical Diseases
University of Pretoria
Tel: 012 529 8265
E-mail: henriette.vanheerden@up.ac.za

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Dear Dr van Heerden,

Your application sent with the email on 15 July 2016 requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following study, "*Prevalence and characterisation of selected pathogens in slaughter animals at Gauteng Province abattoirs – Food safety implications for meat consumers and zoonotic risks posed to abattoir workers*" with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The study must be conducted in compliance with the Veterinary and Para-Veterinary Professions Act 1982 (Act No. 19 of 82) and the Meat Safety Act 2000 (Act No. 40 of 2000);

3. This section 20 approval covers the relevant sub-sections of the main research proposal as declared by the researcher, and does not extend beyond the scope of the specified protocol;
4. Samples must be packaged and transported in accordance the National Road Traffic Act, 1996 (Act No. 93 of 1996);
5. Any incidence or suspected incidence of a controlled or notifiable disease in terms of the Animal Diseases Act 1984 (Act no 35 of 84), must be reported immediately to the State Veterinarian of the area.
6. All potentially infectious material utilised or generated during or by the study is to be destroyed at completion of the study;
7. Only a registered waste disposal company may be used for the removal of waste generated by or during the study;
8. Records must be kept for five years for audit purposes.
9. No part of this study may commence until valid ethical approval has been obtained in writing from the relevant authority;
10. A dispensation for the storage of serum, bacterial isolates and extracted DNA is attached.

Title of research/study: *"Prevalence and characterisation of selected pathogens in slaughter animals at Gauteng Province abattoirs – Food safety implications for meat consumers and zoonotic risks posed to abattoir workers"*


Researcher: Dr Henriette van Heerden

Institution: Department of Veterinary Tropical Diseases, University of Pretoria

Our ref Number: 12/11/1/1/6

Your ref: FY 2015/2016

Kind regards,



DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2016-09-09



agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6

Dr Henriette van Heerden
Department of Veterinary Tropical Diseases
University of Pretoria
Tel: 012 529 8265
E-mail: henriette.vanheerden@up.ac.za

RE: DISPENSATION ON SECTION 20 APPROVAL IN TERMS OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) FOR: "PREVALENCE AND CHARACTERISATION OF SELECTED PATHOGENS IN SLAUGHTER ANIMALS AT GAUTENG PROVINCE ABATTOIRS – FOOD SAFETY IMPLICATIONS FOR MEAT CONSUMERS AND ZONOTIC RISKS POSED TO ABATTOIR WORKERS"

A dispensation is hereby granted on Point 10 of the Section 20 approval that was issued for the above mentioned study (attached):

- i) Serum samples collected from the respective slaughter animals at the specified abattoirs may be stored in the relevant ARC-OVI and UP serum banks;
- ii) Bacterial isolates and extracted DNA obtained from the collected samples may be stored in the relevant ARC-OVI and UP serum banks;
- iii) Stored samples may not be outsourced or used for further research without prior written approval from DAFF.

Kind regards,

DR. MPHO MAJA
DIRECTOR: ANIMAL HEALTH
Date: 2016-09-09