A SEROLOGICAL SURVEY: Q FEVER AMONG ABATTOIR WORKERS IN FREE STATE AND NORTHERN CAPE, SOUTH AFRICA, 2018

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A Research Report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science (Epidemiology).

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DECLARATION

I, Liesl De Boni, declare that this research report is my own original work. Where another person's work has been used (either from a printed source, internet or any other source), it has been acknowledged and referenced in accordance with the guidelines of the Wits School of Public Health, University of the Witwatersrand. I have not used work previously produced by another student or any other person to hand in as my own. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work. This research has not been submitted previously for any other degree or examination at any other institution.

Liesl De Boni

Signed:

Date:

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

This work was exhibited at the following conferences:

It was presented as a scientific poster at the Wits School of Public Health Research day on 22 August 2019 and was awarded the prize for the best poster in the category 'Occupational and Environmental Health'.

It was presented as a scientific poster at the annual conference of the Public Health Association of South Africa, at the College of Cape Town, Athlone, 16 – 18 September 2019.

It was given as an oral presentation at the European Scientific Conference on Applied Infectious Disease Epidemiology, in Stockholm, 27-29 November 2019.

ABSTRACT

Background

Q fever is a ubiquitous bacterial disease, originating from livestock and affecting individuals working with animals/animal products such as farm, veterinary, abattoir or laboratory workers. Infection usually occurs following inhalation of the *Coxiella burnetii* bacteria either in aerosols (close contact with infected animals or the bacteria) or in contaminated dust (spreads more distantly). The spectrum of symptoms ranges from mild flu-like illness to serious complications, e.g. infective endocarditis or chronic fatigue. Little is known of the prevalence of Q fever in South African abattoir workers. This study aimed to estimate the seroprevalence of Q fever and examine factors associated with seropositivity in a sample of abattoir workers in South Africa's Free State and Northern Cape regions.

Methods

This study was a secondary analysis of data from a cross-sectional zoonotic disease survey of abattoir workers conducted in 2018. Previous infection with Q fever was determined using an enzyme-linked immunosorbent assay test for IgG antibodies against *C. burnetii* phase II antigen. Survey logistic regression methods, for clustering and sampling fraction of the data, were then employed to estimate seroprevalence and correlates of seropositivity. Potential confounders such as age, sex and former occupational exposure were adjusted for in the multivariable analysis. A sensitivity analysis was conducted to confirm if the individuals with equivocal test results differed from analysed individuals.

Results

382 abattoir workers were sampled from 16 facilities. The proportions of workers who reported wearing gloves, masks and protective eyewear when performing activities with high risk of QF transmission were 26-35%, 10-17% and 2-7% respectively. Overall, Q fever seroprevalence was 33% (95% confidence interval: [28-38%]), but facility-level seroprevalence ranged between 8 and 62%. The multivariable logistic regression model suggested that contact with carcasses/meat products for the whole day (AOR 4.66 [1.51 – 14.41], p 0.011) and prior work experience at an abattoir/butchery (AOR 1.89 [1.13 – 3.17], p 0.019) were associated with seropositivity. In contrast,

increasing age (AOR 0.96 [0.94 – 0.98], p <0.001) and livestock ownership (AOR 0.32 [0.15 – 0.71], p 0.008) were associated with seronegativity. The sensitivity analysis confirmed that all the individuals with equivocal test results were similar to the rest of the group.

Discussion & conclusion

A high Q fever seroprevalence was detected. The study findings suggest that exposure may occur at the workplace and that workers who spend all day in contact with carcasses/meat products could be at higher risk. We recommend increased precautions to protect the health of personnel who work all day in contact with carcasses/meat products and health education for all abattoir staff.

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NOMENCLATURE

Abattoir lifespan	Indicates how long an abattoir facility has been operational for. The variable is categorized as old (≥22 years) or young (<22 years), where 22 years was the median years of facility establishment in the dataset.
Abattoir size	Refers to the number of staff employed per facility, i.e. small (≤38 staff) or large (>38 staff) – based on the median of staff per facility in the dataset.
Abattoir throughput	Red meat abattoirs are classified as high or low throughput in accordance with the Meat Safety Act (No. 40 of 2000). This is based on the number of animals that they process per day.
Acute disease	A disease or illness that comes on rapidly and lasts for a short time.
Aerosol	A suspension of an infectious agent in the air.
Antibody	A blood protein that is produced by the immune response against a foreign threat to the body (infection).
Antigen	A type of protein that antibodies in the body recognise as foreign.
Cell-mediated immunity	Immune response that does not involve antibodies and works via activation of specific cells, which respond to the antigen directly.
Equivocal test result	This is an inconclusive laboratory test result. It is neither positive nor negative. Ordinarily for a clearer answer, one would take a subsequent specimen from the same subject but that was not possible in this scenario.
Febrile	Having a fever i.e. a body temperature of 38.0°C or above.
Humoral immunity	Immune response mediated by extracellular molecules, e.g. antibodies
Immunoglobulin	Refers to an antibody.
Livestock	Refers to farm animals.
Phagocyte	A type of white blood cell that destroys foreign agents detected in the body by engulfing them internally and destroying them with enzymes.
Prevalence	The proportion of diseased people/animals in a specified population group, expressed as %.
Pseudo-outbreak	When an outbreak is falsely suspected, based on elevated antibody levels, but there are no true cases.
Reservoir host	A group of animals in which a pathogen is maintained and from which infection is transmitted to the target host. Reservoir hosts do not show signs of disease.
Ruminant	A hoofed mammal that chews the cud regurgitated from the rumen. E.g. cattle, sheep, goats, antelopes.
Serology tests	Diagnostic identification of antibodies in serum.
Seropositive	A positive result, using a serological test, indicating the presence of antibodies to a specified infectious agent (antigen).
Seroprevalence	The prevalence of detectable antibody levels against a specified infectious agent.
Serum	The amber-coloured fluid component of blood after the blood clot is removed.
Small stock	Refers to sheep and goats.
Test sensitivity	The ability of a test to correctly identify positive specimens. Expressed as %.
Test specificity	The ability of a test to correctly identify negative specimens. Expressed as %.
Titre	Indicates the concentration of antibodies in blood.
Ubiquitous	Describes something that is found everywhere.
Zoonosis	A disease, which is transmitted from animals to humans.

CHAPTER 1 - Introduction

This chapter serves to inform and orientate the reader about the major concepts of Q fever disease. These concepts are followed by a critical review of the literature about Q fever seroprevalence, risk factors and the use of protective clothing. The chapter closes by introducing the aims and objectives of the study presented in this research report.

The chapter sub-headings are:

1.1 Background

1.1.1 Q fever disease

Aetiology

Q fever, or query fever, is a febrile zoonotic disease that is found worldwide [1, 2]. It is caused by a Gram-negative obligate intracellular bacterium called *Coxiella burnetii* [3]. Q fever is considered highly infectious because, through a pseudo-sporulation process, it is capable of surviving for a long time in the environment, can cause infections at a low infective dose and can be transmitted by the airborne route [4-6]. Once *Coxiella* spores have contaminated an environment, they can cause outbreaks by airborne spread, travelling up to 18 km in strong winds [7]. Q fever often features on various biological threat lists because of its environmental stability and high infectiousness [4, 6]. This bacterium was originally grouped with the Rickettsiae because of its intracellular nature and tick reservoirs [3]. However, since the advent of genetic sequencing, it is now placed within the Legionellaceae order [8]. *Coxiella burnetii* has developed mechanisms to evade the immune system and survive inside the phagocytes within the body. This feature allows it to linger within the body and reactivate to cause persistent infection at a later stage [2].

Epidemiology

Animals infected with Q fever appear well and show no obvious clinical signs. Increased occurrence of spontaneous abortions is often the only warning sign of infection with Q fever in livestock. Although domestic ruminants (cattle, sheep and goats) are the most commonly recognised sources of human infections, parturient domestic animals (cats and dogs) can also be sources of infection. Many other animals (wildlife, rabbits, rodents, fish, reptiles and birds) and arthropods (ticks) are reservoirs. Infected animals shed the bacteria in their urine, faeces, birth products and milk. Humans usually become infected after they inhale the bacteria either within contaminated dust (as a spore-like form) or as aerosolised particles released from birth products (most common), urine, blood, or faeces. Transmission through ingestion of contaminated raw milk is another possible route of infection, although rare [2, 6, 9]. Originally, ticks were thought to play a significant role in the transmission of Q fever [3]. Although numerous tick species are reservoirs of *C. burnetii*, tick bites are no longer considered a major route of transmission of Q fever to humans [10] and there is debate that the presence of the bacteria in ticks merely reflects the prevalence of the bacteria in the animal hosts on which they feed [11]. New research has identified that ticks contain a diverse array of '*Coxiella*-like bacteria', which are genetically similar (related) but distinctly different bacteria than *C. burnetii*. These *Coxiella*-like bacteria form part of the tick natural microbiome and are not infectious for people [12]. Some have suggested that they may have

been misidentified as *C. burnetii* in older field studies and that they could cause false-positive serological test results in people, but these ideas still need to be appropriately investigated.

The first human Q fever infections resembled a 'flu-like' illness and were described in abattoir workers in 1935 in Brisbane, Australia [13]. Males, females and people of all ages are probably equally exposed to the pathogen, but epidemiological studies have found that children and pregnant women are usually asymptomatic or experience milder disease [2, 9, 14]. Acute Q fever is often a non-severe disease and 60% of human infections are asymptomatic. However, acute infection is of concern to public health practitioners as it may progress rapidly to more serious syndromes such as pneumonia, hepatitis, meningitis, osteomyelitis and obstetric complications [1, 8, 9].

Different clinical syndromes predominate in different parts of the world [15]. In Australia, influenza-like illness has been found to be most common. In most of France, southern Spain and Ontario, Canada, more hepatitis is reported. In Crete, Greece, northern Spain, most of Switzerland and Nova Scotia, Canada, pneumonia is more common [15]. It is unclear whether these differences are due to reporting issues or disease dynamics. Possible reporting issues include varying case definitions and selection bias of the cases referred to physicians who publish the results. Disease dynamics that could be responsible are strain virulence factors, routes of infection or inoculating dose of bacteria [15]. As mentioned, *C. burnetii* has an alarming ability to persist intracellularly within the body and cause medical problems later in life, the most frequently reported condition being infective endocarditis. Underlying heart valve disease, being male and older than 40 years are known risk factors for developing this type of infective endocarditis [2, 9]. Observational studies have reported a link between Q fever and chronic fatigue syndrome [2, 15]. The relationship between chronic illnesses and acute Q fever is unclear, little has been published on the subject and some conflicting results have been reported. Though one paper described HIV infection as a risk factor for symptomatic Q fever disease [16], others disagreed [2, 17]. Isolated works were published on the lack of association between Q fever and diabetes, corticosteroid therapy [18] and immunosuppressive therapy [19] but more research is needed to further elucidate some of these relationships.

Q fever is not routinely tested for nor is it a notifiable disease in many countries so the infection rates in both humans and animals are not well-established, and this is critical to understanding the potential risks to humans [10, 20]. A One Health approach is recommended for the control of Q fever disease, meaning that there should be collaboration between the human, veterinary and environment health sectors [21, 22]. Domestic ruminants are most often the source of Q fever infections in humans, thus control of *C. burnetii* in animal reservoirs will reduce Q fever in the human population by reducing human exposure [22].

Control and prevention

A brief overview of Q fever control and possible prevention methods are described here. Broadly, the possible approaches for Q fever control may be grouped into four measures:

1) Identification of infected farms. This could be done by notification of the symptoms of Q fever, e.g. increased abortions, or positive test results [22].
2) Reduction of *C. burnetii* excretion. In some countries, Q fever positive farms use animal vaccination and institute breeding bans until the disease is under control [22]. During a large outbreak in the Netherlands in 2007-2010, all Q fever-infected pregnant animals were culled to stop the excretion of more bacteria and animal vaccination was instituted [9, 23].
3) Reduction of the dispersion of *C. burnetii*. The bacteria may be dispersed in the form of infected animals or their products. Transport of animals from positive farms should be controlled or banned depending on the situation [22]. The spreading of manure from infected herds should not be allowed [9]. Livestock births in infected herds could also be managed indoors and the birth products could be safely removed (and disposed of) and the areas disinfected afterwards [22].
4) Reduction of human exposure. Human exposure could be minimised by avoiding direct contact between humans and infected animals. Visitors should not be allowed to visit infected farms [22]. The milk from infected farms should be discarded [9]. Persons who must work with infected animals or people should take special precautions [2]. The live *C. burnetii* bacteria should only be handled in a biosafety level (BSL) 3 laboratory by experienced operators. Veterinary and healthcare personnel who will knowingly be exposed to infected patients should wear protective respiratory masks and gloves. For the best clinical outcomes in people, early diagnosis and treatment of the infection is desired. Clinicians should maintain increased suspicion for Q fever among persons with unexplained fever that are from high-risk occupational groups [2].

Individuals from high-risk occupational groups (veterinary workers, farmers, abattoir workers, laboratory personnel) experience potential regular exposure to *C. burnetii* [2, 24, 25]. One of the ways to protect these individuals is human vaccination, as is done in Australia. Due to low initial uptake of the vaccine, the Australian Government funded a national Q fever vaccination program that was rolled out in 2001 targeting high-risk occupational groups [26]. The inactivated vaccine used offers good protection. A meta-analysis reported that vaccinated personnel working in abattoirs experienced 96% reduced risk of Q fever infection (risk ratio 0.06 [0.02–0.18]). The most common adverse event reported was injection site reaction [25]. A stringent screening process is recommended prior to vaccination because individuals with pre-existing immunity to Q fever may experience severe reactions to the vaccine. This is both time-consuming and costly. Vaccine availability can be another barrier for many countries [9]. By improving

the understanding of exposure to Q fever among South African abattoir workers, decisions about which control measures to implement could be better informed.

Serological diagnosis

Under-diagnosis of Q fever probably occurs due to the high proportion of asymptomatic cases (60%) and because the symptoms may be mild and non-specific [8]. Q fever tests are not routinely requested or available, and the bacteria should only be cultured in laboratories with biosafety level 3 conditions [2]. Therefore, it is challenging to make a clinical diagnosis of human Q fever and confirmation often relies on serological blood tests [27]. Fournier *et al* described many serological methods but those that are the most reliable and commonly used are indirect immunofluorescence (IFA), enzyme-linked immunosorbent assay (ELISA), complement fixation, and microagglutination. Ideally, serological epidemiological tests should have high specificity to minimise false-positive results [27]. A good understanding of Q fever serology is necessary to avoid misinterpretation, causing a pseudo-outbreak [28].

Serology is the study of antibodies (immunoglobulins) in serum, which are proteins formed in response to infection. A positive serological test indicates the body's response to an infection. At least a four-fold increase in antibody titre is needed to prove a current or recent infection [2, 27, 29]. It is impossible to make this distinction from a single positive serology test result. At best, a single positive test means that there is evidence of exposure but there is no information about whether the immune response is recent or not. Thus, laboratories recommend testing a second serum sample two to three weeks later. Q fever bacteria display antigenic variation (phase I and II antigens), and different antibodies are stimulated at different stages of the disease [Figure 1.1]. This property is useful to differentiate between acute and chronic illness [2, 27, 29].

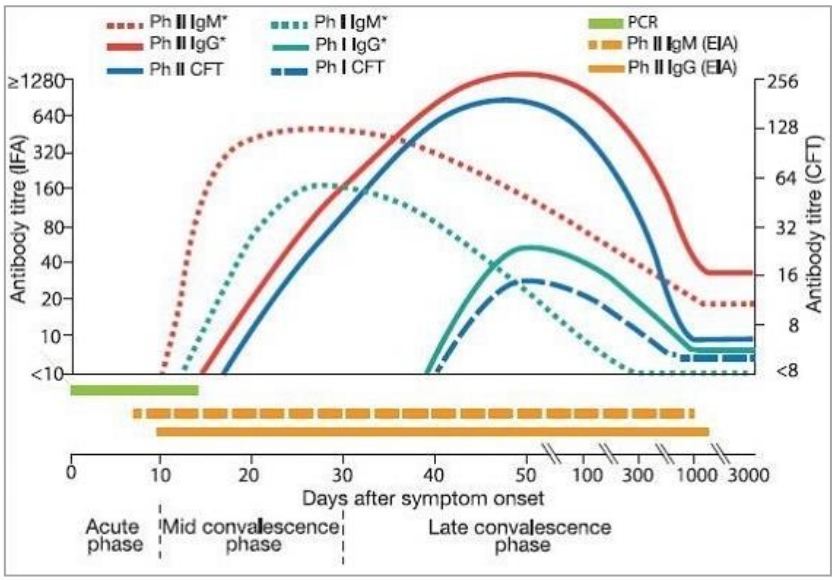

Figure 1. 1. Schematic diagram depicting the typical antibody responses during human Q fever [29].

In general, immunoglobulin type M (IgM) antibody levels rise from two weeks after infection and decrease after 4 months. Immunoglobulin type G (IgG) usually peak around 4-8 weeks after the onset of illness and they can stay elevated for years [14, 15]. In acute Q fever, phase II antibodies (IgM2 and IgG2) reach the highest levels, whereas persistent Q fever is characterised by high levels of phase I antibodies [21, 27]. After acute Q fever, IgG2 have the longest half time of all the antibody types, estimated as 2.6 years in a Dutch study [30]. Normally in the absence of re-exposure, antibody titres decrease over time eventually becoming undetectable [31]. However, evidence suggests that continuous exposure to *C. burnetii*, in occupational settings, has a 'boostering effect' on Q fever antibodies [32, 33] and this could result in persistently high titres long-term. The study described in this research report tested for IgG2 to detect past Q fever infections (or previous exposure) to *C. burnetii.*

In addition to the humoral (antibody-mediated) immune response, a full cell-mediated immune response is needed to overcome a Q fever infection [2]. Cell-mediated immunity is briefly mentioned here because it is an important aspect to take note of - serological tests are unable to detect it. To our knowledge, there is no reliable blood test for cell-mediated immunity against Q fever yet. There is an intradermal skin test for this purpose. It involves subcutaneous injection of a low dose of the Q fever vaccine and re-evaluation for a skin reaction after seven days [9, 15]. The vaccine and trained personnel for performing the test are not available in many countries including South Africa, so intradermal testing is not possible in all situations [9].

Seroprevalence and distribution

Over the years, seroprevalence studies of Q fever in Africa have yielded scattered and varying results in both humans and animals [11, 17]. No easy diagnostic tools are available in most African countries so the public health impact of the disease is largely underestimated [2]. The comparison of prevalence studies is challenging because many used convenience sampling methods and the serological tests (and cut-off values) were not standardised [17]. More systematic reviews and comprehensive studies of the molecular epidemiology in Africa are needed [11].

Reported human seroprevalence across Africa is variable [11, 17]. Figures range between 1% in Chad and 32% in Egypt [2, 11], with seroprevalence being observed in Mali, Burkina Faso, Nigeria, and Central African Republic [2]. Other recent studies revealed seroprevalence of 26% among blood donors in Namibia, 15-30% in Algeria and 25% in rural areas of Senegal [2]. Higher seroprevalence, of 8-17%, was reported among children in Ghana, Niger and the Gambia [11]. In general, increased seropositivity coincided with countries of high domestic ruminant densities and living in rural areas [2, 11, 17]. In neighbouring Zimbabwe, a 37% (n=494) human seroprevalence was reported [34]. In general, reported seroprevalence in African domestic ruminants was high. In the literature, seroprevalence in animals was 4-55% in cattle, 13-24% in goats, 11-33% in sheep, 23% in dogs and 70-80% in camels [11, 17].

The first human case of Q fever in South Africa was reported by Gear and colleagues in 1950 [35] and a second case was reported soon after by Ranking [36]. Prior to that, it was considered a disease of academic interest, endemic to Queensland, Australia, and Montana, U.S.A [35, 36]. In the following years, Q fever was found to be as common as tick-bite fever by a dedicated investigation of human cases in South Africa [37]. Most South Africans, especially in rural areas, seemed to be immune and it was thought that they acquired the infection (and immunity) in early childhood [38]. The majority of cases at the time were seen in recently-arrived overseas immigrants and their South African-born children. Gear expressed a concern that Q fever might become a bigger problem in the future because, due to trends of rapid urbanisation and reduced exposure to reservoir animals, the proportion of susceptible people in the population could increase [37, 38]. By 1986, Q fever was considered endemic to the former Transvaal Province and about 8% of 8900 cattle had positive antibody titres [39]. However, a much wider distribution of the disease was suggested by detection of the agent in foetal and placental tissues of sheep and cattle on twelve different farms covering a large area in southern Africa [40]. One serological study suggested that domestic cats might play a role in Q fever epidemiology in southern Africa but the sample sizes were small – one in 52 (2%) South African and 15 in 113 (13.3%) Zimbabwean cats [41].

Two recent South African studies demonstrated significant seroprevalence of Q fever among two separate groups of healthy people working with animals in Mpumalanga Province. Both studies were cross-sectional and tested for IgG2 antibody. They found that 61% of 64 healthy persons working at state-operated cattle dip tanks [42] and 33% of 258 people working in the Kruger National Park [43] were seropositive. This indicates that Q fever is still prevalent in Mpumalanga Province.

1.1.2 The public health importance of Q fever

Q fever is a public health issue because outbreaks affecting the public can potentially be large and cause significant public healthcare problems as a result. This was demonstrated by the Netherlands outbreak [21]. Several papers have identified residents of rural or agricultural areas as having a higher risk of Q fever infection [17, 44-46]. This is probably because human exposure to Q fever depends on the density of infected animals [11] and animal numbers are generally higher in rural versus urban areas. Immunity after Q fever infection has been described as life-long [47], and infection acquired during childhood is often asymptomatic or mild [9]. Thus, people living in rural/agricultural settings and people who are regularly exposed to Q fever are possibly more often immune and protected from the disease. Individuals with no underlying immunity are especially at risk. This includes visitors or immigrants to endemic areas and people who do not have regular contact with livestock [2, 21, 38]. The main factors that drove the Q fever outbreak in the Netherlands were the simultaneous presence of 1) a large population of susceptible people (urban dwellers), 2) high levels of *C. burnetii* bacteria in the environment due to ongoing outbreaks on nearby dairy goat farms, and 3) the ideal weather conditions for the bacteria to spread [21, 23].

Although outbreaks of Q fever in humans may not be common, it is important to learn from them. A summary of most of the largest outbreaks published in the last 30 years is shown [Table 1.1]. The top-ranking outbreak, mentioned earlier, resulting in over 4000 notified cases in the Netherlands, deserves a mention. Investigators estimated that each notified human case represented 12 new cases implying that approximately 44000 people were infected, and exposed to the risk of persistent Q fever infection. The presence of Q fever probably went undetected for some time due to under-diagnoses and under-reporting. Investigations could not identify a single source for the outbreak. There was strong evidence that the major source of the outbreak was multiple infected goat farms situated nearby densely populated urban areas. A few infections were acquired from a particular infected sheep farm. Rodents might also have assisted in wider spreading of the contamination [21]. The presence of *C. burnetii* in air was demonstrated and the seasonal pattern of cases corresponded with the animal birthing season [4]. This outbreak caused unprecedented public healthcare problems demonstrated by: high hospitalisation rates, challenges for

simultaneous management by veterinary and human health authorities, and management of transmission risk due to blood donation by asymptomatic infected individuals. With such a large number of people infected, the economic and health implications were immense. Prompt and drastic veterinary measures eventually controlled the outbreak. Nevertheless, it is likely that Q fever will remain a problem in the Netherlands for years to come because the bacteria were widespread in both the environment and animal reservoirs [21, 23, 48, 49]. This enormous outbreak occurred in urban communities and did not discriminate by job type, contrary to other outbreaks [48].

It is clear by looking at table 1.1 that the largest Q fever outbreaks were not confined to rural areas and that small stock holdings were a common source of infections. It is interesting that although outbreaks in rural populations were the most frequent, outbreaks in urban populations were among the largest – taking the top six positions. Large outbreaks in occupationally-exposed groups were reported less frequently but two of them affected almost 100 people each. The suspected sources of these outbreaks were primarily small stock farms, followed by unknown, farms in general and environmental contamination. The determination of the animal source of these outbreaks was mainly based on circumstantial evidence, e.g. simultaneous detection of the disease in humans and animals. Proper source confirmation, however, requires special molecular techniques to link the human cases to an animal source - this was often not done. The study presented here used abattoir workers as a group to enlighten the public health sector as to whether Q fever could pose any possible danger to people in South Africa.

Table 1. 1. A summary of human Q fever outbreaks resulting in ≥20 cases, published in the last 30 years.

Country	Group affected, place	Type	Year	Cases detected	Suspected source of outbreak
Netherlands	Residents, southeast regions [21]	U	2007-2010	4 026 (notified)	Multiple sources. Small stock (mainly goats) were the main source. Rats might have had a smaller role in spreading the contamination
Germany	Residents, Jena [50]	U	2005	331	Flocks of gestating sheep nearby
Bulgaria	Residents, Botevgrad [51]	U	2004	220	Herds of small stock nearby
UK	Residents, West Midlands (England) [52]	U	1989	147	Nearby farmland
Israel	Students & staff at a boarding school, Tel Aviv [53]	U	2005	144	Dining room air-conditioning system
Italy	Prisoners, prison staff & residents, Como [54]	U	2003	133	Flocks of sheep grazed nearby
Slovakia	Residents, Jedl'ove Kostol'any [55]	R	1993	113	Herds of imported goats that had abortions. Animal workers that often visited the local pub wearing contaminated clothes might have played a role
Scotland	Staff at an abattoir & cutting plant [56]	O	2006	110	Sheep holding pen at the abattoir
UK	Staff at a cardboard manufacturing plant, Gwent (Wales) [57]	O	2002	95	Unknown. Possibly due to drilling into contaminated straw boards during renovations
Hungary	Residents, Baranya county [58]	R	2013	70	Large flock of merino sheep nearby. Sequence typing confirmed
Canada	Farmers, workers & their contacts, Newfoundland [59]	R	1999	66	Aborting goats on eight affected farms. Manure spread as fertiliser may have contributed
Spain	Staff at a waste sorting plant, Basque Country [60]	U	2014	62	Exposure occurred inside the plant. Animal remains within the sorting residues
Spain	Residents, north Spain [61]	R	2003	60	Unknown. Prevalent area
Italy	Residents, Vicenza [62]	U	1993	58	Flocks of sheep nearby
Germany	Residents, Rollshausen [63]	R	1996	49	Sheep flock nearby
Serbia	Residents, Vojvodina [64]	R	2012	43	Unknown
Germany	Residents, Freudenstadt [65]	R	2008 2009	41 29	A new large flock of sheep nearby & contamination of pastures
Slovenia	Students & staff at a veterinary high school, south-west area [66]	O	2007	35	Attendance of a training course on a sheep farm
USA	Residents & ranchers at a horse boarding ranch (Colorado) [67]	R	2005	31	Two new goat herds introduced at the ranch
UK	Residents, Cheltenham (England) [68]	U	2007	30	Sheep farms nearby
France	Residents, Alps [69]	R	1996	29	Uncovered sheep waste in the slaughterhouse area. Possibly facilitated by nearby heliport
Bosnia	Soldiers in a Czech army unit [70]	R	1997	26	Flock of parturient sheep nearby. Possibly facilitated by nearby heliport
Australia	Farmers & residents, South Australia [71]	O	2004	25	Sheep at a large sheep market
Iraq	Soldiers in an US marines unit, west Iraq [72]	R	2005	22	Unknown
Spain	Attendees & staff at a day care centre for the mentally handicapped [73]	O	-	22	School farm visit
Israel	Residents, Haifa [74]	U	2001	21	Unknown. Possibly livestock facilities nearby
USA	Staff at seventeen goat farms (Washington, Montana & Oregon) [75]	O	2011	21	Multiple goat herds nearby
Not specified	Soldiers in an Yugoslav army unit [76]	R	-	20	Unknown. Possible entry into sheep-dense area during lambing season

*U = urban, R = rural, O = occupational. Investigators used various diagnostic tests for case detection.

1.1.3 Abattoir workers as an important risk group

Abattoir workers are recognised as an occupational group with increased risk of acquiring various zoonotic infections due to their close contact with animal products. This is particularly true for Q fever [24, 25, 77, 78]. Several papers have demonstrated high exposure to various zoonoses in people who slaughter animals or who work in abattoirs [26, 79-85]. Public health practitioners are concerned about the health of abattoir workers. Abattoir workers are closely connected to the human food chain and they can act as sentinels for prevalent zoonotic infections [77]. Indeed, they may be the first to present with previously unrecognised diseases, as was the case with Q fever [13]. Therefore, abattoir workers can serve as an early warning system for outbreaks of zoonotic infections such as Q fever.

A study of Malaysian abattoir workers reported physical, chemical, biological, psychosocial, musculoskeletal, and ergonomics as major occupational hazards. It stressed the importance of both good abattoir design and good environmental hygiene to manage these issues [86]. By law, South African employers have a duty to their staff to provide a safe work environment [87]. The Occupational Health and Safety Act (No. 85 of 1993) as amended by the Occupational Health and Safety Amendment Act (No. 181 of 1993) provides for the health and safety of all persons at work. Section 8 states that, within reason, an employer should: create and maintain an environment that is safe and without risk to the health of employees; take steps to eliminate/mitigate any threats to this safety; provide the necessary instructions, training and supervision to employees to ensure this safety; and enforce these measures [87]. It is important to remember that health can be threatened by infectious diseases. Therefore, the Act applies to protection from any biological threats (e.g. *C. burnetii* bacteria) that employees may encounter at work too. This concept was introduced in Australia where, with government support, more and more abattoirs implemented Q fever vaccination programmes at the workplace [15]. There are also several independent companies that offer Q fever screening and vaccination of workers in Australia [88].

In the South African market, there are occupational health medical services for the abattoir industry, and abattoir workers must be certified medically fit before assuming their duties [89]. Section 17 of the Occupational Health and Safety Act (No. 85 of 1993) as amended by the Occupational Health and Safety Amendment Act (No. 181 of 1993) declares that any workplace with more than 20 employees, or when an inspector deems necessary, must designate health and safety representatives. The chosen representatives liaise with the employer and Act in all matters pertaining to health and safety at work on behalf of all the staff [87]. They should implement the safety measures that they deem to be necessary based on the risk

profile of the abattoir and its staff. Thus, different facilities prioritise different hazards and not all abattoirs may have programs in place for the infectious disease hazards that are present.

Protecting abattoir workers from Q fever is not only ethical, it also makes economic sense. Infections among staff can cost abattoir employers both due to sick leave taken and the potential litigation implications. A median of 3 weeks of annual sick leave has been reported for illness due to Q fever and time to full recovery can be prolonged, with a median of 12 weeks [90]. Complicated cases may even result in long-term complications and up to 6 months sick leave being claimed. Although the link between Q fever and chronic fatigue is debated by some [91], Australian courts have granted large pay-outs to employees for this condition [15]. If a high level of exposure to Q fever is found in the study from this report, appropriate preventive measures could be implemented to protect this high-risk group.

1.2 Literature Review

1.2.1 Literature search strategy

Between July 2018 and September 2019, regular online searches were conducted to find studies published in English that discussed the seroprevalence of human Q fever, risk factors associated with seropositivity and the identification of any protective factors. A local expert and two international experts on Q fever were also contacted directly (in person and via email) to extract further studies. The online databases used were PubMed and Google Scholar. The various search terms used were: (‘Q fever’ OR ‘Query fever’ OR ‘Coxiella’ OR ‘coxiellosis’ OR ‘burnetii’ OR ‘burneti’) AND (‘prevalence’ OR ‘seroprevalence’ OR ‘risk factor’ OR ‘risk’ OR ‘incidence’) AND/OR (‘abattoir worker’ OR ‘abattoir’ OR ‘meat worker’ OR ‘meat’ OR ‘slaughterhouse’ OR ‘butcher’ OR ‘occupation’ OR ‘farmer’ OR ‘veterinarian’). Different permutations of these terms were also employed. After selecting the relevant literature from the abstracts, the full text papers were uploaded into and managed using EndNote. The main criteria for presentation of studies in this literature review were that they had to be observational studies or systematic reviews about human Q fever, in people working in high-risk occupations, which reported seroprevalence and associated risk or protective factors. Outbreak investigations that revealed relevant risk/protective factors were kept. Arthropod vector studies, case reports, case series, textbooks/manuals and experimental studies were excluded.

1.2.2 Seroprevalence of Q fever among high-risk occupational groups

Human Q fever prevalence differs greatly between countries depending on whether or not the disease is notifiable. In endemic areas, cases usually occur sporadically following at-risk activities such as farming, slaughter work or rural tourism [2]. Thus, low seroprevalence is typically observed in the public and higher seroprevalence is usually expected in at-risk occupational groups. The studies that looked at the seroprevalence of Q fever among healthy individuals in at-risk occupational groups are discussed in this section. Comparison between studies was challenging because some either did not specify the *C. burnetii* phase antigen or they combined antibody phase types [46, 80, 92-100] and some used non-ELISA serological tests [46, 79, 80, 83, 97, 98, 100-105]. Where phase I and phase II antibody results were reported separately [81, 82, 101, 105, 106], the value for phase II was used. Where multiple serological tests were used and reported separately [96, 107], the results for ELISA tests were used. The studies that used ELISA to test for IgG2 were the most similar to the Q fever study presented in this research report [42, 43, 81, 82, 106, 108-110].

Some seroprevalence studies sampled veterinarians, farmers, farm workers, butchers/abattoir workers, laboratory staff, hunters, game rangers or field guides and support staff working in animal-dense environments as a homogenous group [43, 81, 92, 93, 101, 106, 107, 109]. It may not be accurate to group all of these occupations together as their exposure risks for Q fever may not be truly comparable. These papers revealed seroprevalence ranging 4-63% in this mixed group, but if one only looks at the studies that had sample sizes larger than 200, then the seroprevalence range narrows to 4-37% [Table 1. 2].

Farmers, farm workers and livestock veterinarians, who have more similar exposure risks for Q fever, were frequently studied in several recent papers [42, 46, 94-100, 103, 104, 110]. Broadly, seroprevalence ranged 4-89% but if one only considers studies with sample sizes over 200, then the estimate range was 11-36% [Table 1. 2].

Several studies of Q fever seroprevalence among abattoir workers alone were found [79, 80, 82, 83, 102, 105, 108]. The most recent papers came from South Korea and Iran [82, 83, 102, 108], some of the studies recruited small samples sizes [79, 80, 82, 105, 108] and some were published more than 26 years ago [79, 80, 105]. However, all of the papers that were found are described here since they discuss the group of interest in this research report. Once again, a wide range of Q fever seroprevalence was reported, 9-30%. It is also interesting to note that similar levels of pre-existing immunity to Q fever were reported in Australian abattoir workers prior to the implementation of the national Q fever vaccination program, for abattoir

workers, in 2001. They found that 29% (n=485) and 34% (n=1751) of abattoir workers in New South Wales and Queensland respectively demonstrated evidence of prior Q fever infections [26] [Table 1. 2].

Table 1. 2. Seroprevalence estimates among at-risk occupational groups for Q fever.

Group	Country	Seroprevalence	Sample size	Year
Veterinarians, farmers, farm workers, butchers./abattoir workers, laboratory staff, hunters, field guides and people working in animal conservation parks	Trinidad [92]	4%	455	2011
	Canada [107]	13%	40	2018
	Thailand [93]	13%	661	2017
	Western Iran [81]	15%	250	2014
	Western Iran [106]	26%	637	2019
	South Africa [43]	33%	258	2014
	Iran [101]	37%	401	2017
	Sicily [109]	63%	140	2015
Farmers, farm workers and livestock veterinarians	Ecuador [94]	4%	32	2019
	Korea [103]	11%	1222	2018
	USA [95]	22%	508	2009
	China [104]	31%	362	2016
	Poland [96]	39%	151	2015
	Belgium [97]	45%	108	2017
	India [110]	46%	93	2018
	Canada [98]	59%	32	2017
	South Africa [42]	61%	64	2018
	Netherlands [46]	65%	189	2013
	India [99]	89%	19	2019
	Netherlands [100]	72%*	755	2014
Butchers/abattoir/meat workers only	South Korea [102]	9%	923	2018
	South Korea [83]	10%	1481	2017
	Japan [105]	11%	107	1993
	Iran [82]	14%	190	2016
	Scotland [79]	28%	96	1966
	Brazil [80]	29%	144	1975
	Iran [108]	30%	144	2019
	Australia [26]			2009
	- New South Wales	29%	485	
	- Queensland	34%	1751	

A random effects meta-analysis of Q fever seroprevalence among abattoir workers tried to overcome the difficulties around between-country comparisons [84]. The analysis included 19 papers and was stratified by outbreak status, year, country and serological technique used. An overall seroprevalence of 26% (95% confidence interval [18 - 35%]) was estimated in this high-risk group. The slaughtering of cattle, sheep and goats were reported as the major risk factors for seropositivity and for development of symptomatic Q fever. Individual age and years of occupational experience were not significantly associated with seropositivity. Even though the papers included in the analysis were declared to be sufficiently homogenous to be pooled together into a single prevalence measure, it must be noted that several papers were published long ago (52 years ago), different serological tests were used, and the antibody types detected were not specified [84].

1.2.3 Risk factors for Q fever seropositivity in high-risk occupational settings

Most of the literature about the risk factors for Q fever comes from outbreak investigations, analyses of notification data and serological surveys. The most commonly reported risk factors for Q fever seropositivity are being male, age, contact with livestock and living in rural areas. Although people of both sexes and all ages are equally exposed to Q fever, seropositivity is observed more frequently in males and persons older than 40 years because they developed clinical disease more often [48, 111-113]. Contact with livestock may occur either generally [17, 90, 111, 114, 115] or occupationally among farmers [42, 99, 100, 103, 104], abattoir workers [82, 84, 106, 108] and livestock veterinarians [46, 95, 98]. Living in rural areas is most likely a risk factor because usually these are the areas with the highest numbers of livestock [2, 17, 46, 106, 116].

A number of risk factors that are relevant in an abattoir setting were identified in the literature. The acts of slaughtering [117] or evisceration [102] of animals and working with their skins/hides [3] are associated with increased risk of Q fever exposure. On the contrary, a large study of Q fever in slaughterhouse workers in South Korea (n=1481) demonstrated no statistical difference in seroprevalence between workers in the slaughter areas versus residual product handlers. They did however find that regular contact of cattle blood around the mouth was a risk factor for seropositivity in slaughterhouse workers [83]. Similarly, a study among 1222 dairy cattle workers showed that contact of birth products with the eyes or mouth was a risk factor for Q fever seropositivity [103]. Live animals can also transmit Q fever at the abattoir by stirring up dust in the holding pens and not only inside the facility. This was demonstrated by a study of 144 Brazilian abattoir workers that reported Q fever seroprevalence according to work activity. The seroprevalence was highest among staff working in the animal holding pens being exposed to dust and hides (40%), followed by staff working on the killing floor being exposed to blood and viscera (36%) and then staff handling meat in the deboning (20%) or sausage (14%) sections [80]. It is also supported by two outbreak investigations that identified specific live animal holding areas as the outbreak sources. One was a large outbreak at a Scottish slaughterhouse and they found that the sheep holding area was most likely the source [56]. The other outbreak occurred in Australia, and they found that the cases probably became infected when they visited a sheep sale yard on a specific windy day [71].

Some other interesting risk factors that were identified in the literature are briefly mentioned here. A number of papers describe contact with manure or being nearby when manure was spread as a risk factor for Q fever [97, 115, 118]. Smoking was also reported as a risk factor for Q fever in two studies [48, 99]. A study among butchers and abattoir workers found that slaughtering camels was associated with increased seropositivity [82]. This is interesting because high seroprevalence for *C. burnetii* was observed in this

species and contact with camels is reported as a risk factor for Q fever in Africa [11, 17]. This might indicate that camels are a high-risk species.

1.2.4 Protective factors for Q fever seropositivity in high-risk occupational settings

Several examples of possible protective factors and preventative measures were identified in the literature and are described here [2, 60, 81, 99, 100, 102, 104, 119]. This is important because if a significant level of Q fever exposure is detected among abattoir workers in the study presented here, then some of these concepts may be used to help protect this group.

During an outbreak of Q fever that affected 58% of 106 employees at an urban waste sorting plant in Spain, the staff who wore respiratory protection masks while working were significantly protected compared to those who did not. These masks were filtering face pieces class 2 or above, which have a total filtering efficiency of >92% following the European standard EN149:2001 [60]. In another example, office staff working at an Australian goat farm were the most affected during an outbreak of Q fever. The staff working inside the factory on the farm were better protected than the office staff because the factory had high-efficiency particulate arrestance (HEPA) air filtration systems that protected the factory workers from airborne spread of the bacteria on the farm [119]. These findings highlight the importance of respiratory protection against Q fever infection, which is most often acquired by inhalation [2]. No evidence was found in the literature that clearly states the minimum filtering efficiency that is required of any mask to be adequately protected from *C. burnetii* on an infected farm or at an abattoir. Laboratory protocols recommend BSL3 precautions and N95 protective masks are advised for healthcare personnel who are treating parturient women diagnosed with Q fever or who must perform autopsies on patients suspected to have died of Q fever [2].

A number of studies identified general hygiene and disinfection practices that appeared to be protective for Q fever seropositivity. One such practice was compliant use of gloves either when cleaning cattle excrement [103] or when assisting with calf birth care [100]. Washing ones hands and face after work [81] and good personal/hand hygiene [99] was another protective practice identified. Having disinfection equipment on dairy cattle farms was yet another protective factor for Q fever seropositivity [104]. Therefore, hygiene practices and protective clothing may be used to protect at-risk people from being exposed to *C. burnetii* [2, 60, 81, 99, 100, 102, 104, 119].

1.3 Problem statement

Q fever has been well described and much is known about the disease. Its existence in South Africa has been recognised since 1950 [35, 36]. Despite this, very little new information has been produced in the South African context. Only two recent studies demonstrated the continued presence of the bacteria in Mpumalanga Province [42, 43]. Even though much has been published about Q fever among abattoir workers in other countries, no investigations in abattoir workers or other provinces in South Africa were found. Not knowing the extent of Q fever exposure among high-risk groups may lead to inadequate public health education and protection from occupational infection. The human seroprevalence of Q fever in South Africa might be lower now than it once was but the true prevalence remains unknown and the disease is largely under-diagnosed in both humans and animals.

1.4 Justification

Research on Q fever has emerged over the last 80 years. However, several key questions remain for the field [120]. These include questions about human susceptibility factors, pathogen virulence factors, diagnostic tools and new therapeutic strategies [2]. About 10 years ago, a large outbreak in the Netherlands sparked renewed interest and some important Q fever work is underway as a result [21]. This study set out to understand if Q fever could be a threat to an at-risk occupational group in South Africa.

By raising awareness of Q fever in South Africa, the findings of this study could aid improvement of safety practices at abattoirs, prompt research for deeper understanding of the issues and stimulate changes in policies for Q fever control.

1.5 Research question

What are the factors associated with Q fever seropositivity among abattoir workers in South Africa between March and May 2018?

1.6 Aims & Objectives

This study aimed to estimate the seroprevalence of Q fever and determine the factors associated with past infection among abattoir workers in the Free State and Northern Cape provinces, South Africa. The objectives of the study were to:

i. Compare the individual and facility level characteristics of sampled abattoir workers who were seropositive with those who were negative for Q fever.
ii. Estimate the seroprevalence of Q fever in a sample of abattoir workers in the Free State and Northern Cape provinces, South Africa.
iii. Determine exposure factors associated with Q fever seropositivity among abattoir workers in the Free State and the Northern Cape Province.

CHAPTER 2 - Methods

This chapter gives a brief background of the primary study including the sampling strategy and data collection. The methods behind the data management and analysis of the secondary data analysis for Q fever are then described.

The chapter sub-headings are:

2.1 The primary study

The primary study was a project entitled “Towards a better understanding of Rift Valley Fever and Brucellosis Epidemiology in the Republic of South Africa”. It was a collaborative project between the National Institute for Communicable Diseases (NICD), EcoHealth Alliance and several other stakeholders. The project sought to understand the factors that drive re-emergence of RVF virus [121-123], to estimate the true seroprevalence and to explore risk factors associated with previous RVF virus and brucellosis infections in humans and animals [85, 124] in the South African context.

The abattoir survey was a sub-study that was nested within the primary study. It aimed to investigate several zoonotic diseases among abattoir workers, namely Q fever, Rift Valley fever (RVF), Crimean-Congo haemorrhagic fever (CCHF) and brucellosis.

2.1.1 Study design

The abattoir study was designed as a cross-sectional study design.

2.1.2 Study setting

The RVF project was conducted between April 2014 and May 2019. It covered a specific 40 000 km^2 area between Bloemfontein and Mokala National Park, predominantly the Free State and parts of the Northern Cape provinces [Figure 2. 1]. This area was selected by the RVF project because it was affected by RVF virus outbreaks in the past [125].

The abattoir survey targeted all functioning abattoirs located within the primary study area [Figure 2. 1] and was conducted between 13 March and 27 May 2018.

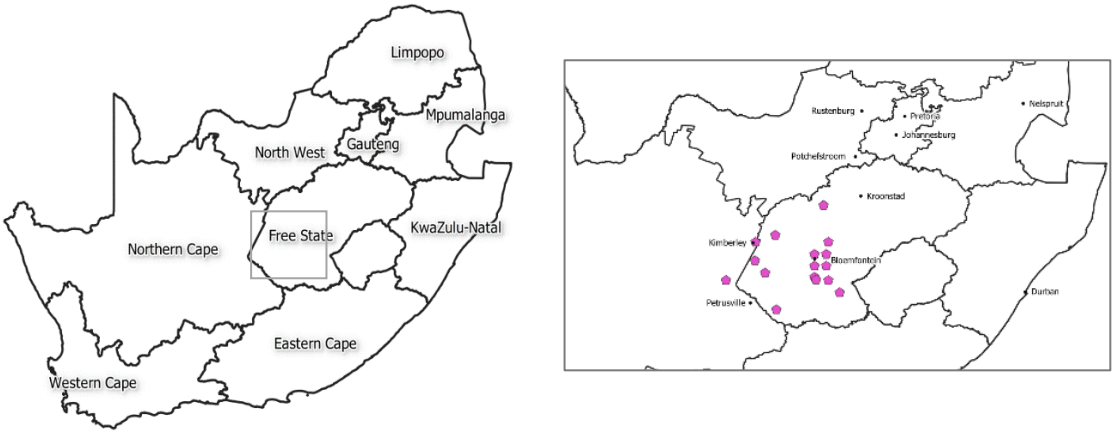

Figure 2. 1. Locations of sampled abattoirs (right) within the study area (left) in the abattoir survey, South Africa, May 2018. Image created in QGIS (http://qgis.osgeo.org) using coordinates collected in the field.

2.1.3 Study population

The study population was all consenting abattoir workers in the study area.

2.1.4 Sampling strategy

Of the 28 listed abattoir facilities, 17 were found to be functioning. All functional abattoir facilities were included in the abattoir survey. At each consenting facility, individuals were sampled using a purposive sampling strategy. In other words, all abattoir workers were allowed to participate but the number sampled was limited by the time available. In the larger abattoirs, those who performed slaughtering and who handled carcasses were prioritised for sampling.

2.1.5 Field data collection procedures

The following procedure was followed at each sampled facility during the abattoir survey. To minimise the economic implications for facility owners and the potential consequences for animal welfare, it was a priority to cause the least possible interruption to production processes. The research team spent one or two days at each facility, depending on the number of staff interested in participating and the approval of facility management.

After granting written informed consent for the employees to participate [Appendix D], the facility owner or manager answered a pre-designed facility questionnaire [Appendix F]. A trained interviewer assisted

and the data were captured electronically on a mobile device. The facility questionnaire collected operational data such as: the date of establishment, daily number of animals processed, animal species processed, source of animals processed, types of equipment used and hygiene procedures followed. After granting written informed consent for their participation [Appendix E], individual participants completed a pre-designed electronic questionnaire [Appendix G]. Consent forms were provided in their preferred language (English, Afrikaans or Sesotho) and a trained interviewer was available to assist with any problems. The questionnaire collected demographic, health-related, knowledge, practices and exposure data. Finally, a professional nurse collected a blood specimen (approximately 17 ml) from all consenting participants. The blood specimens were centrifuged daily on site to obtain serum. The serum specimens were transported, while refrigerated, to the Centre for Emerging Zoonotic and Parasitic Diseases (CEZPD) at the National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Service (NHLS). There they were divided into aliquots and stored frozen at -20.0°C until testing.

The electronic questionnaire did not capture individual identifying information. At the time of consent, a sequential anonymous participant ID number was generated. This number was captured in the electronic questionnaires and was used to label the blood specimens. Only the assigned researcher, Veerle Msimang, had access to the linking information.

The serum aliquots were transferred to the Special Bacterial Pathogens Reference Laboratory, within the CEZPD, for Q fever testing in December 2018. After the serum specimens were thawed and mixed well, they were tested for IgG2 using a commercial ELISA test (Vircell, Granada, Spain). The antigen, *C. burnetii* phase II Nine Mile (ATCC VR-616), and the necessary controls were provided with the test kit. The steps of the assay procedure were followed as specified by the manufacturer. For each specimen, the antibody index was calculated [(sample optical density/cut-off serum mean optical density) x 10] and interpreted as per the manufacturer's guidelines: <9 negative, 9-11 equivocal and >11 positive. All equivocal specimens were retested following the same procedure. Any specimens that had an equivocal result on the second test remained equivocal.

2.2 Study design

The secondary data analysis under taken for this research report is a cross-sectional study design.

2.3 Sampling strategy

All records of abattoir workers who were tested for IgG2 antibodies against *C. burnetii* were included in the current study. This was 382 individuals out of approximately 1350 abattoir workers employed in the study area.

2.4 Precision & power calculation

The sample size for the secondary analysis of the Q fever data was determined at the outset by the abattoir survey. Thus, no sample size calculations were made. Nevertheless, the precision (for a significance level of 5%) and power of the study were calculated from the available data. Precision of 4.6% was calculated manually using the formula below, where p was the seroprevalence and n was the sample size. This means that the seroprevalence estimates varied by this amount and was reflected in the 95% confidence intervals.

$$Precision = \pm 1.96\sqrt{\frac{(p)(1-p)}{n}} \times 100\% = \pm 1.96\sqrt{\frac{(0.3)(0.7)}{382}} \times 100\% = 4.59\%$$

The power calculation was calculated in Stata (StataCorp, College Station, TX, USA) using the power-calculator [126]. The personal ownership of livestock, a binary exposure variable, was selected from the dataset. The absolute number and the seroprevalence proportions of individuals who reported owning or not owning livestock, were obtained from the dataset and entered into the power-command. A power of 85.8% was calculated.

2.5 Data collection

Two datasets were used for the Q fever study. The survey data, that were collected by questionnaires in the field, was obtained from the primary study. The second dataset comprised of the final Q fever test results was provided by the laboratory.

2.6 Data management

The cleaned survey data were received in a Microsoft Excel worksheet and imported into Stata version 15 (StataCorp, College Station, TX, USA). The final Q fever results, also in Excel, were imported and merged with the survey data using the anonymous participant ID number. The total number of staff employed at each facility were added manually.

The first objective was to describe the study participants by serological status. In addition to the sociodemographic characteristics, other individual and facility characteristics as well as usage of protective clothing at work were used. Based on knowledge from the literature review, studies that suggested working with live animals, performing slaughter/evisceration and skinning [3, 80, 102, 117] and all cleaning activities were considered activities with potentially higher risk of Q fever exposure in this analysis. The process of cleaning was added because it brings individuals closer into contact with possibly infectious materials (e.g. animal blood or excreta), and the cleaning action could aerosolise any bacteria present within a closed area.

The second objective was carried out using the outcome variable, seropositivity to IgG2. The possible values were positive or negative. Equivocal results were handled as missing and therefore excluded from this analysis.

The third objective required both the outcome variable and the exposure variables of interest in the Q fever study. The exposure variables of interest to the Q fever study were extracted from the data for this purpose. The methods and reasoning behind the re-organisation of existing exposure variables for this analysis are provided [Supplementary 1].

2.6.1 Exposure variables

The exposure variables of interest that were analysed are listed here, but more details are given in the supplementary section [Supplementary 2]:

Sociodemographic variables
- Age
- Sex
- Population group
- Education

Facility-level variables
- Abattoir throughput
- Abattoir size
- Abattoir lifespan
- Animals sourced from individuals for personal consumption
- Animals sourced from private farms
- Animals sourced from feedlots
- Animals sourced from community farms
- Animals sourced from auctions
- Slaughter pigs
- Slaughter sheep only
- Slaughter cattle
- Personnel required to wear masks (in general)
- Personnel required to wear gloves (in general)
- Personnel required to wear goggles/face shields (in general)

Individual-level variables
- Previous work experience
- Daily duration of direct contact with carcasses/meat products
- Work schedule
- Period of employment
- Wearing protective/sanitary clothing at work (in general)
- Receiving/holding live animals
- Live animal clinical examination
- Slaughter, evisceration and/or carcass dressing
- Product packaging
- Freezing finished products
- Processing hides
- Treating wastewater
- Transporting processed material
- Cleaning equipment
- Cleaning slaughter areas
- Cleaning outside of slaughter areas
- Carcass/meat inspection
- Other close animal/product contact
- Any sharp injury while working with a condemned product
- Any tick bites
- Consumption of undercooked meat
- Current treatment for any chronic illness
- Current treatment for rheumatoid arthritis
- Current treatment for heart disease

Current treatment for asthma
Current treatment for immunosuppression (e.g. HIV, immunosuppressive medication, malignancy)
Current treatment for tuberculosis
Current treatment for renal disease
Current treatment for other chronic disease
Personal ownership of livestock

The variables of concurrent treatment of chronic illness(es) were used as a proxy for concurrent chronic disease. As this is a prolific sheep farming region, all sampled facilities processed sheep. Thus, slaughtering of sheep could not be analysed as a factor associated with seropositivity. Certain other exposures could not be analysed because they were unique to a single facility and had no comparison group. These were animals being sourced by importation, animals being sourced for ceremonial slaughter or the processing of goats, poultry and wild antelope. Some other exposure variables could not be analysed because none of the participants with these characteristics were also seropositive for Q fever. These were less common non-communicable diseases, namely liver disease, obesity and diabetes.

2.6.2 Outcome variable

As per the result from the laboratory, the outcome could either be negative, positive or equivocal. Equivocal results were excluded from the analysis.

2.7 Descriptive analysis

2.7.1 Study participants

Categorical variables were described by the number and percentage of individuals in each category. The continuous variables were not normally distributed, so they were described by giving the median and interquartile range.

2.7.2 Seroprevalence estimates

The true seroprevalence estimates were calculated using Blaker's method [127]. This method adjusts the proportions of seropositive specimens for the Q fever ELISA test characteristics. The sensitivity and

specificity of the test used were 95% and 97% [128] when it was compared to the IFA test, the reference test for Q fever serology [2].

To accommodate the characteristics of the study design used to collect the data, the apparent seroprevalence estimates were calculated using Stata version 15 (StataCorp, College Station, TX, USA). Since participants were sampled in clusters by abattoir, larger between-abattoir variability was expected than if individual-level sampling had been done. This increased variability negatively influences the precision of any analysis of clustered data. To account for this problem, the linearised variance estimator function, which is based on a first-order Taylor series linear approximation [129], was used. This was achieved by first specifying the data as survey data, clustered by abattoir facility using the svyset-command. All subsequent estimations and hypothesis testing commands were preceded by the svy-prefix. The relative proportions of staff sampled per facility (sampling fraction) differed because the number of staff employed varied and the time available for data collection was limited. Consequently, participants working at small facilities had a higher probability of being sampled than participants working at larger facilities. This bias was accounted for with probability weights. This is when weights that are proportional to the inverse probability of being selected, are applied to the individual observations [130]. They were calculated by dividing the total number of staff working at a facility by the number of staff sampled at the facility. The probability weights were specified at the same time the svyset-command, prior to analysis.

Apparent seroprevalence estimates were calculated as the proportion of positive specimens using the proportion-command. This was repeated for facility characteristics of abattoir size, throughput and lifespan. Facility-level seroprevalence was also estimated using the proportion-command but these did not account for the study design since facility was the clustering variable. Nevertheless, this was done to demonstrate the wide range of facility-level seroprevalence. The true and apparent seroprevalence estimates were compared.

2.8 Inferential analysis

2.8.1 Univariable analysis

Univariable analysis was conducted by looking for associations between the outcome and each exposure variable individually [131]. After specifying the clustering and sampling fraction of the data, simple logistic regression was employed for each exposure variable of interest using Stata version 15 (StataCorp,

College Station, TX, USA). All exposure factors with a p value of under 0.2 were potentially significant and considered candidates for inclusion in the multivariable model.

2.8.2 Multivariable analysis

There were two levels to the data used in this analysis, namely individual-level and facility-level. A multivariable logistic regression analysis, adjusted for clustering and sampling fraction, was chosen instead of a multilevel statistical model. This was because these data did not have higher level units and the logistic regression would not require reliance on such strong assumptions as are necessary for multilevel models [132]. The selection of the final regression model followed the approach recommended by Victora and colleagues and was similar to that used by Kinyanda and colleagues [133, 134]. A simplified version of their conceptual hierarchical framework of risk factors for infectious diseases in developing countries [Supplementary 3] was adapted for the purposes of this study [134]. In this analysis, the sociodemographic variables were treated as distal determinants, the facility-level exposure variables were treated as intermediate determinants and the individual-level exposure variables were treated as proximal determinants of Q fever seropositivity. The potential bias that could be introduced by confounders or study design was mitigated by including all associated covariates identified in the literature during the regression model building. Specifically, these were age and sex [2, 111, 112].

The selection of the final multivariable logistic regression model (model 4) was completed in three stages using Stata version 15 (StataCorp, College Station, TX, USA). Backward elimination was used at each stage. Model 1 assessed associations between Q fever seropositivity and the sociodemographic variables (distal determinants). Covariates were eliminated using a backward stepwise method with a lenient p value threshold of 0.2. Any sociodemographic variables selected here were kept in all subsequent models [133, 134]. Model 2 assessed associations between Q fever seropositivity and those facility-level exposure variables (intermediate determinants) that were candidates based on the univariable analysis. Exposure variables were eliminated using a backward stepwise method but this time using a slightly stricter p value threshold of 0.1. Model 3 examined associations between Q fever seropositivity and those individual-level exposure variables (proximal determinants) that were candidates based on the univariable analysis. Again, exposure variables were eliminated using a backward stepwise method with the p value threshold of 0.1. All variables that were selected in model 2 and 3 were carried into the final model, model 4. Prior work experience was kept in the final model as this was expected to behave as a possible confounder. Those variables with a p value of less than 0.05 were considered statistically significant.

After the final model (model 4) was fitted, a post-regression goodness-of-fit test was used to confirm if the model accurately described the true outcome observed by the data. This was achieved with the svylogitgof-command, which estimates the F-adjusted mean residual test after the svy logistic estimation has been carried out. This test accounts for the clustering and sampling weights [135].

Finally, the exposure variables remaining in the final logistic regression model were reported. Both the unadjusted odds ratios, from the univariable analysis, and the adjusted odds ratios, from multivariable analysis, are presented in the results chapter.

2.8.3 Sensitivity analysis

To assess if the individuals with equivocal results differed significantly from the analysed individuals, a sensitivity analysis was conducted. The sensitivity analysis comprised two stages. First, the equivocal results were treated as positive and second they were treated as negative. The same analysis procedure described above was repeated for the two stages using the two sets of results. The outcomes of the final logistic regression models (model 5 and 6 respectively) were reported and compared with model 4.

2.9 Responsibilities of the student

The student, Liesl De Boni, was not involved in the primary study. However, she visited each of the abattoir facilities and provided individual feedback to the participants during May 2019. At the same time, she collected additional data that were necessary for this analysis. Advice was sought from a statistician regarding the approach to the analysis, but the student was responsible for managing the data, creating the necessary variables and conducting the analyses.

2.10 Ethical considerations

The existing ethics approval for the RVF project, from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, was amended in November 2017 to include the abattoir survey, clearance certificate no. M140306. The Q fever study obtained ethical approval from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand in October 2018, clearance certificate no. M180959 [Appendix B].

CHAPTER 3 - Results

This chapter begins by describing the study participants' characteristics and protective clothing usage. Next, the seroprevalence estimates for Q fever are reported. The exposure factors that correlated with Q fever seropositivity in the univariable and multivariable analysis are then reported. Finally, the findings of the sensitivity analysis are laid out.

The chapter sub-headings are:

3.1. Study participants

3.1.1. Characteristics

In total, 382 abattoir workers were sampled and thirteen did not complete the questionnaire, so although their blood was tested their exposure data was missing. Thus, most participants were male (72%, 265/369), black African (83%, 306/369), and had attained an education level of up to secondary school (80%, 294/369). The median age of all participants was 35 years (interquartile range: 29 - 42 years). There were 250 individuals with negative results, 109 with positive results and 23 with equivocal results [Table 3. 1].

Table 3. 1. Sociodemographic characteristics and Q fever test results of participants in the abattoir survey, South Africa, May 2018

		Negative group (N=250)		Seropositive group (N=109)		Equivocal group (N=23)	
Age (years)	Median [IQR]	35 [30 - 42]		33 [27 - 38]		32 [27 - 40]	
Sex (n=369)	Female	69	29%	31	29%	4	21%
	Male	173	71%	77	71%	15	79%
Population group[†] (n=369)	Black African	198	82%	91	84%	17	89%
	Coloured	37	15%	16	15%	1	5%
	White	7	3%	1	1%	1	5%
Education (n=369)	None	10	4%	4	4%	1	5%
	Primary	30	12%	11	10%	1	5%
	Secondary	193	80%	85	79%	16	84%
	Higher	9	4%	8	7%	1	5%

IQR: interquartile range, N: total number in the group, n: number with complete data.

Most of the participants were full-time employees (96%, 351/364) and worked inside the abattoir (93%, 343/369). Those who did not work inside the abattoir worked either in administration or as drivers. The median period of employment at the abattoir was 5 years (interquartile range: 3 – 10 years). About one-third participants had prior experience working in the abattoir/butchery industry (27%, 101/368) and only 17 % (62/368) had prior work experience in farming. The majority of individuals reported not owning any livestock personally (84%, 310/368), with ownership of cattle (10%, 38/368) followed by sheep (6%, 21/368) being most reported. The most frequent four work tasks performed by participants were cleaning abattoir equipment (77%, 293/382), cleaning slaughter areas (74%, 282/382),

[†] The population groups 'White', 'Black African', 'Indian/Asian' and 'Coloured' were created during the former South African Apartheid system. People with ancestors from India/Asia were called 'Indian/Asian'; people with mixed ancestry were called 'Coloured'. These categories are still used in census and statistical data today. The terms are only used in this report to investigate possible differences in health between these population groups, and not to support the former sociocultural categories.

slaughter/evisceration/carcass dressing (55%, 209/382) and cleaning other areas (55%, 209/382). Most of the workers sampled performed more than one task and very few reported performing a single task exclusively. Out of 368 workers, those who did a single task were: five doing slaughter/evisceration/carcass dressing, one packaging meat products, one doing water treatment, one doing transport, two cleaning equipment, three cleaning the slaughter area, four cleaning outside the slaughter area and one taking microbiological specimens. A quarter of the participants reported being on chronic treatment (25%, 91/366), with the most common chronic illness treated for being tuberculosis (30%, 27/91) followed by immunosuppressive conditions (24%, 22/91), renal disease (16%, 15/91), and rheumatoid arthritis (12%, 11/91). For the full wording of the questions, the questionnaire was supplied [Appendix G]. The exposure variables of interest and their serological status are shown [Table 3. 2].

Table 3. 2. Exposure factors and Q fever test results of participants in the abattoir survey, South Africa, May 2018

Exposure variable		Negative group (N=250)		Positive group (N=109)		Equivocal group (N=23)	
FACILITY-LEVEL							
Abattoir throughput	Low	20	8%	7	6%	0	0%
	High	230	92%	102	94%	23	100%
Abattoir size	Small (0 - 38 staff)	89	36%	34	31%	3	13%
	Large (> 38 staff)	161	64%	75	69%	20	87%
Abattoir lifespan	Young (≤22 years)	137	55%	66	61%	14	61%
	Old (> 22 years)	113	45%	43	39%	9	39%
Animals sourced from individuals for personal consumption	No	70	28%	41	38%	10	43%
	Yes	180	72%	68	62%	13	57%
Animals sourced from private farms	No	28	11%	15	14%	6	26%
	Yes	222	89%	94	86%	17	74%
Animals sourced from feedlots	No	196	78%	82	75%	16	70%
	Yes	54	22%	27	25%	7	30%
Animals sourced from community farms	No	130	52%	58	53%	11	48%
	Yes	120	48%	51	47%	12	52%
Animals sourced from auctions	No	49	20%	22	20%	3	13%
	Yes	201	80%	87	80%	20	87%
Slaughter pigs	No	184	74%	88	81%	20	87%
	Yes	66	26%	21	19%	3	13%
Slaughter sheep only	No	211	84%	95	87%	19	83%
	Yes	39	16%	14	13%	4	17%
Slaughter cattle	No	44	18%	18	17%	5	22%
	Yes	206	82%	91	83%	18	78%
Personnel required to wear a mask	No	76	30%	29	27%	6	26%
	Yes	174	70%	80	73%	17	74%
Personnel required to wear gloves	No	37	15%	20	18%	1	4%
	Yes	213	85%	89	82%	22	96%
Personnel required to wear goggles	No	81	32%	37	34%	7	30%
	Yes	169	68%	72	66%	16	70%
INDIVIDUAL-LEVEL							
Previous work experience	Other	139	57%	56	52%	10	53%
	Farming	43	18%	17	16%	2	11%
	Abattoir/butcher	60	25%	34	32%	7	37%
Daily duration of contact with carcass/meat products	< 1 hour/day	15	6%	2	2%	1	5%
	< half day	27	11%	6	6%	1	5%
	Whole day	200	83%	99	92%	17	90%
Period employed	median [IQR]	6 [3 – 12]		5 [3 – 10]		3.5 [3 – 8]	
Work schedule	Part time	10	4%	3	3%	0	0%

Exposure variable		Negative group (N=250)		Positive group (N=109)		Equivocal group (N=23)	
	Full time	229	96%	103	97%	19	100%
Wearing protective clothing at work	Regular/always	24	10%	8	7%	2	11%
	Sometimes/never	218	90%	99	93%	17	89%
Receiving/holding animals	No	172	71%	78	73%	16	84%
	Yes	70	29%	29	27%	3	16%
Live animal clinical examination	No	217	90%	92	86%	17	89%
	Yes	25	10%	15	14%	2	11%
Slaughter, evisceration and/or carcass dressing	No	106	44%	43	40%	10	53%
	Yes	136	56%	64	60%	9	47%
Packaging meat products	No	134	55%	67	63%	13	68%
	Yes	108	45%	40	37%	6	32%
Freezing finished products	No	139	57%	68	64%	12	63%
	Yes	103	43%	39	36%	7	37%
Processing hides	No	180	74%	72	67%	16	84%
	Yes	62	26%	35	33%	3	16%
Treating wastewater	No	158	65%	71	66%	13	68%
	Yes	84	35%	36	34%	6	32%
Transporting processed material	No	173	71%	83	78%	17	89%
	Yes	69	29%	24	22%	2	11%
Cleaning equipment	No	49	20%	21	20%	5	26%
	Yes	193	80%	86	80%	14	74%
Cleaning slaughter areas	No	61	25%	21	20%	4	21%
	Yes	181	75%	86	80%	15	79%
Cleaning outside of slaughter areas	No	103	43%	46	43%	10	53%
	Yes	139	57%	61	57%	9	47%
Collecting microbial samples	No	212	88%	93	87%	17	89%
	Yes	30	12%	14	13%	2	11%
Carcass/meat inspection	No	208	86%	89	83%	15	79%
	Yes	34	14%	18	17%	4	21%
Other close animal/product contact	No	213	88%	98	92%	17	89%
	Yes	29	12%	9	8%	2	11%
Sharp injury while working with condemned product	No	129	54%	53	50%	6	33%
	Yes	112	46%	54	50%	12	67%
Tick bite	No	183	76%	79	74%	14	78%
	Yes	58	24%	28	26%	4	22%
Eat undercooked meat	No	138	57%	68	64%	10	56%
	Yes	103	43%	39	36%	8	44%
Treatment for any chronic illness	No	172	71%	87	81%	16	89%
	Yes	69	29%	20	19%	2	11%
Rheumatoid arthritis	No	61	88%	17	85%	2	100%
	Yes	8	12%	3	15%	0	0%
Treatment for heart disease	No	58	84%	16	80%	2	100%
	Yes	11	16%	4	20%	0	0%
Treatment for asthma	No	66	96%	19	95%	1	50%
	Yes	3	4%	1	5%	1	50%
Treatment for immunosuppression	No	53	77%	14	70%	2	100%
	Yes	16	23%	6	30%	0	0%
Treatment for tuberculosis	No	48	70%	14	70%	2	100%
	Yes	21	30%	6	30%	0	0%
Treatment for renal disease	No	58	84%	16	80%	2	100%
	Yes	11	16%	4	20%	0	0%
Treatment for liver disease	No	68	99%	20	100%	2	100%
	Yes	1	1%	0	0%	0	0%
Treatment for obesity	No	67	97%	20	100%	2	100%
	Yes	2	3%	0	0%	0	0%
Treatment for diabetes	No	65	94%	20	100%	2	100%
	Yes	4	6%	0	0%	0	0%
Treatment for other chronic illness	No	232	96%	104	97%	17	94%
	Yes	9	4%	3	3%	1	6%
Livestock ownership	No	196	81%	99	93%	15	79%
	Yes	46	19%	8	7%	4	21%

Almost a third (28%, 382/1350) of the abattoir workers in the study area were sampled from 16 different operating facilities. Although one facility in the study area declined to participate. Most of the facilities were in the Free State Province, and two were in the Northern Cape Province. The facility sizes varied from 6 employees up to 520. Abattoirs with over 100 staff ranked lowest in terms of participation [Table 3. 3]. Almost all of the participants worked in high-throughput facilities (93%, 355/382) and more worked in facilities with a large number of employees (67%, 256/382) than not. Although all of the individuals worked at facilities that processed sheep, a large proportion also processed cattle (82%, 315/382); fewer also processed pigs (24%, 90/382) and a minority (less than 3%) processed other species such as goats, poultry and wild antelope.

Table 3. 3. Size and level of participation of facilities in the abattoir survey, South Africa, May 2018

Abattoir facility	Number sampled	Number employed	% sampled (participated)
A	6	6	100%
B	9	10	90%
C	33	38	86%
D	5	7	71%
E	35	51	69%
F	9	14	64%
G	33	54	61%
H	22	38	58%
I	20	35	57%
J	12	29	41%
K	26	78	33%
L	10	33	30%
M	47	172	27%
N	27	159	17%
O	15	101	15%
P	73	520	14%

Overall, the records were reasonably complete with few missing data. The level of missing data did not exceed 4% for any of the variables of interest.

3.1.2. Protective clothing

The practices of wearing masks, protective eyewear and gloves ordered by number of workers partaking in the work activity were summarised [Table 3. 4]. Only 10 – 17% of participants reported using masks when performing tasks with potentially higher risk for Q fever exposure and only 2 – 7% reported using protective eyewear. However, the reported compliance for using gloves while performing these tasks was higher, between 26 and 35%.

Table 3. 4. Usage of masks, protective eyewear and gloves by participants in the abattoir survey, South Africa, May 2018

Work activity	n	Mask	Eyewear	Gloves
*Cleaning equipment	293	51 (17%)	20 (7%)	104 (35%)
*Cleaning slaughtering areas	282	44 (16%)	21 (7%)	93 (33%)
*Slaughter, evisceration and/or carcass dressing	209	29 (14%)	10 (5%)	62 (30%)
*Cleaning outside of slaughtering areas	209	33 (16%)	10 (5%)	56 (27%)
Packaging meat products	154	30 (19%)	9 (6%)	51 (33%)
Freezing finished carcasses/products	149	25 (17%)	7 (5%)	44 (30%)
*Treating waste water	126	21 (17%)	4 (3%)	41 (33%)
*Receiving/holding livestock	102	13 (13%)	5 (5%)	29 (28%)
*Processing hides	100	13 (13%)	3 (3%)	26 (26%)
Transporting processed material	95	19 (20%)	2 (2%)	23 (24%)
Carcass/meat inspection	56	8 (14%)	3 (5%)	17 (30%)
Collecting microbial samples	46	6 (13%)	1 (2%)	14 (30%)
*Clinical examination of live animals	42	4 (10%)	1 (2%)	12 (29%)
Other close animal contact	40	12 (30%)	3 (8%)	19 (48%)

*Activities with higher potential risk of Q fever exposure.

3.2. Seroprevalence estimates

Since there were 23 individuals with equivocal results, the results of 359 individuals were included in the seroprevalence estimations. The apparent and true seroprevalence estimates for Q fever in sampled abattoir workers were similar [Table 3. 5]. The true seroprevalence estimates, which accounted for the test characteristics, were more conservative than the apparent seroprevalence estimates, which accounted for the study design characteristics. Overall, Q fever seroprevalence was 30-33% and it followed a wide range between facilities, namely 8-62%. Only smaller, low-throughput and older abattoirs experienced below average seroprevalence.

Table 3. 5. Apparent and true seroprevalence estimates for Q fever in the abattoir survey, South Africa, May 2018

Category	n/N	Apparent sero-prevalence %	95% Confidence Interval	True sero-prevalence %	95% Confidence Interval
Overall	109/359	33	28 - 38	30	25 - 35
Abattoir size					
Small	34/123	27	18 - 39	27	19 - 36
Large	75/236	34	28 - 40	31	25 - 38
Abattoir throughput					
Low	7/27	24	14 - 36	25	11 - 45
High	102/332	33	28 - 39	30	25 - 36
Abattoir time established					
Young	66/203	34	28 – 41	32	26 - 39
Old	43/156	29	22 - 38	27	20 - 35

N = total tested in the group, n: number positive individuals.

3.3. Univariable correlates of seropositivity

The outputs of the univariable analyses for all explanatory variables of interest are presented [Table 3. 6]. The sociodemographic variables of potential significance included age and education level. The individual level exposure variables of potential significance included being treated for any chronic illness, daily duration of contact with carcasses/meat products, freezing of finished products, previous work experience, other close contact with animals or their products, work schedule, transporting of processed materials, wearing of protective clothing at work, slaughter/evisceration/carcass dressing and ownership of livestock. The facility level exposure variables of potential significance included sourcing of animals from individuals for personal consumption or from private farms, slaughtering pigs, slaughtering sheep only, personnel being required to wear a mask at work and abattoir throughput.

Table 3. 6. Factors associated with Q fever seropositivity in the univariable analysis of the abattoir survey, South Africa, May 2018

Exposure variable		n (%)	Unadjusted OR (95% CI)	p value
SOCIODEMOGRAPHIC				
Age (years)			0.96 (0.93 – 0.98)	**0.004**
Education level	None	15 (4%)	Ref	**0.086***
	Primary school	42 (11%)	2.13 (0.25 – 18.11)	
	Secondary school	294 (80%)	3.68 (0.70 – 19.19)	
	Higher education	18 (5 %)	5.25 (0.68 – 40.29)	
Population group	Black African	306 (83%)	Ref	0.236*
	Coloured	54 (15%)	0.70 (0.24 – 2.06)	
	White	9 (2%)	0.12 (0.01 – 1.52)	
Sex	Male (vs female)	265 (72%)	1.11 (0.73 – 1.68)	0.615
FACILITY LEVEL				
Abattoir throughput	High (vs low)	355 (93%)	1.62 (0.83 – 3.14)	**0.143**
Abattoir size	Large (vs small)	256 (67%)	1.37 (0.75 – 2.48)	0.280
Abattoir lifespan	Old (vs young)	165 (43%)	0.79 (0.50 – 1.27)	0.311
Animals sourced from individuals for personal consumption	Yes (vs no)	261 (68%)	0.60 (0.41 – 0.87)	**0.011**
Animals sourced from private farms	Yes (vs no)	333 (87%)	0.62 (0.34 – 1.14)	**0.115**
Animals sourced from feedlots	Yes (vs no)	88 (23%)	1.04 (0.67 – 1.62)	0.841
Animals sourced from community farms	Yes (vs no)	183 (48%)	1.04 (0.66 – 1.64)	0.857
Animals sourced from auctions	Yes (vs no)	308 (81%)	1.03 (0.57 – 1.84)	0.920
Slaughter pigs	Yes (vs no)	90 (24%)	0.61 (0.39 – 0.96)	**0.036**
Slaughter sheep only	Yes (vs no)	57 (15%)	0.71 (0.44 – 1.13)	**0.136**
Slaughter cattle	Yes (vs no)	315 (82%)	1.10 (0.63 – 1.93)	0.722
Personnel required to wear a mask	Yes (vs no)	271 (71%)	1.34 (0.92 – 1.97)	**0.122**
Personnel required to wear gloves	Yes (vs no)	324 (85%)	0.81 (0.55 – 1.18)	0.249
Personnel required to wear goggles	Yes (vs no)	257 (67%)	0.99 (0.60 – 1.62)	0.959
INDIVIDUAL LEVEL				
Previous work experience	Other	205 (56%)	Ref	**0.101***
	Farming	62 (17%)	1.01 (0.48 – 2.11)	
	Abattoir/butcher	101 (27%)	1.86 (0.97 – 3.58)	
Daily duration contact with carcass/meat products	< 1 hour/day	18 (5%)	Ref	**<0.001***

Exposure variable		n (%)	Unadjusted OR (95% CI)	p value
	< half day	34 (9%)	1.41 (0.24 – 8.09)	
	Whole day	316 (86%)	3.52 (1.30 – 9.52)	
Schedule	Full time (vs part time)	351 (96%)	5.06 (0.71 – 36.15)	**0.099**
Period employed (years)			0.98 (0.94 – 1.03)	0.382
Wearing protective clothing at work	Regularly/always (vs sometimes/ never)	334 (91%)	1.75 (0.81 – 3.80)	**0.142**
Receiving/holding live animals	Yes (vs no)	102 (28%)	1.11 (0.53 – 2.33)	0.766
Live animal clinical examination	Yes (vs no)	42 (11%)	1.27 (0.73 – 2.21)	0.372
Slaughter, evisceration and/or carcass dressing	Yes (vs no)	209 (57%)	1.37 (0.87 – 2.16)	**0.166**
Packaging meat products	Yes (vs no)	154 (42%)	0.79 (0.51 – 1.23)	0.270
Freezing finished products	Yes (vs no)	149 (41%)	0.77 (0.61 – 0.98)	**0.035**
Processing hides	Yes (vs no)	100 (27%)	0.77 (0.61 – 0.98)	0.313
Treating wastewater	Yes (vs no)	126 (34%)	1.01 (0.46 – 2.22)	0.987
Transporting processed material	Yes (vs no)	95 (26%)	0.67 (0.41 – 1.09)	**0.101**
Cleaning equipment	Yes (vs no)	293 (80%)	1.07 (0.55 – 2.08)	0.830
Cleaning slaughter areas	Yes (vs no)	282 (77%)	1.23 (0.81 – 1.87)	0.312
Cleaning outside of slaughter areas	Yes (vs no)	209 (57%)	0.87 (0.64 – 1.19)	0.347
Collecting microbial samples	Yes (vs no)	46 (13%)	0.95 (0.52 – 1.74)	0.866
Carcass/meat inspection	Yes (vs no)	56 (15%)	1.23 (0.62 – 2.47)	0.526
Other close animal/product contact	Yes (vs no)	40 (11%)	0.49 (0.23 – 1.07)	**0.071**
Sharp injury while working with condemned product	Yes (vs no)	178 (49%)	1.15 (0.77 – 1.74)	0.468
Tick bite	Yes (vs no)	90 (25%)	1.05 (0.67 – 1.65)	0.823
Eat undercooked meat	Yes (vs no)	150 (41%)	0.92 (0.42 – 2.03)	0.823
Treatment for any chronic illness	Yes (vs no)	91 (25%)	0.68 (0.48 – 0.97)	**0.034**
Treatment for rheumatoid arthritis	Yes (vs no)	11 (3%)	1.93 (0.66 – 5.68)	0.211
Treatment for heart disease	Yes (vs no)	15 (4%)	1.39 (0.48 – 4.08)	0.520
Treatment for asthma	Yes (vs no)	5 (1%)	0.56 (0.04 – 7.71)	0.645
Treatment for immunosuppressive Illness	Yes (vs no)	22 (6%)	1.12 (0.60 – 2.09)	0.700
Treatment for tuberculosis	Yes (vs no)	27 (7%)	1.10 (0.56 – 2.17)	0.768
Treatment for renal disease	Yes (vs no)	15 (4%)	0.83 (0.20 – 3.53)	0.791
Treatment for other chronic illness	Yes (vs no)	9 (2%)	1.35 (0.39 – 4.68)	0.611
Livestock ownership	Yes (vs no)	58 (16%)	0.34 (0.20 – 0.60)	**0.001**

n=number in the category, OR: odds ratio, CI: confidence interval, Ref: reference group.
*The p value was taken for the categorical variable as a whole in the univariable analysis.

3.4. Multivariable correlates of seropositivity

The sociodemographic variable that was selected in model 1 was age. In model 2, the facility level exposure variable selected was animals being sourced from individuals for personal consumption. In model 3, the individual level exposure variables selected were daily duration contact with carcass/meat products, personal ownership of livestock and treatment for any chronic illness [Table 3. 7]. In the final model (model 4), prior work experience and all of the selected variables were included. Model 4 demonstrated a good fit with the data as seen by the post-regression goodness of fit test result of 0.052 (>0.05). The unadjusted and adjusted odds ratios for model 4 are reported [Table 3. 8, Figure 3. 1].

Table 3. 7. Summary of models in analysing factors associated with Q fever seropositivity of the abattoir survey, South Africa, May 2018

Model	Exposure variables included in the model	OR (95% CI)	p value
1	Age	0.97 (0.95 – 1.00)	0.018
	Education level	Eliminated	0.522*
(cut off	Population group	Eliminated	0.513*
p<0.2)	Sex	Eliminated	0.799
2	Age	0.97 (0.95 – 1.00)	0.026
	Animals sourced from individuals for personal consumption	0.62 (0.38 – 1.01)	0.056
	Slaughter sheep only Slaughter pigs	Eliminated	0.452
	Slaughter sheep only	Eliminated	0.482
	Animals sourced from private farms	Eliminated	0.714
(cut off	Personnel required to wear a mask	Eliminated	0.922
p<0.1)	Abattoir throughput	Eliminated	0.984
3	Livestock ownership	0.30 (0.13 – 0.68)	0.004
	Age	0.97 (0.94 – 0.99)	0.016
	Treatment for any chronic illness	0.57 (0.32 – 1.03)	0.061
	Daily duration contact with carcass/meat product		0.073*
	- <1/2 day	2.42 (0.41 – 14.16)	
	- Whole day	4.53 (0.98 – 20.89)	
	Transporting processed material	Eliminated	0.264
	Previous work experience	Eliminated	0.344*
	Schedule	Eliminated	0.396
	Slaughter, evisceration and/or carcass dressing	Eliminated	0.407
	Other close animal/product contact	Eliminated	0.497
(cut off	Wearing protective clothing at work	Eliminated	0.620
p<0.1)	Freezing finished products	Eliminated	0.883

OR: odds ratio (only given for those included in the model, CI: confidence interval.
*The p value was taken for the categorical variable as a whole in the model selection.

Age appeared to have a mild protective effect. For every year increase in age, the odds of being seropositive decreased by 4%. Three individual level exposure factors stood out. Participants with prior work experience at an abattoir/butchery had 89% higher odds of Q fever seropositivity than those without. Compared to those who reported spending less than an hour in contact with carcasses/meat products on a typical workday, abattoir workers who reported spending the whole day in contact experienced a 4.56 odds of Q fever seropositivity. In contrast, personal ownership of livestock demonstrated a protective effect; with livestock owners having 68% reduced odds of seropositivity than non-owners. Treatment for any chronic illness and animals being sourced from individuals who intended to utilise the products for personal consumption, remained in the final model and appeared to be protective factors, but both were no longer statistically significant in the end. This is suggested by their p values, which were 0.139 and 0.114 (>0.05) respectively and the odds ratio confidence intervals that included 1.0 [Table 3. 8].

The equation for the final logistic regression model is provided [Supplementary 4].

Table 3. 8. Factors associated with Q fever seropositivity in the multivariable analysis of the abattoir survey, South Africa, May 2018

Exposure variable	n (%)	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age (years)		0.96 (0.93 – 0.98)	0.004	0.96 (0.94 – 0.98)	**<0.001**
Previous work experience					
Other	205 (56%)	Reference		Reference	
Farming	62 (17%)	1.01 (0.48 – 2.11)		0.86 (0.43 – 1.68)	0.629
Abattoir/butcher	101 (27%)	1.86 (0.97 – 3.58)	0.101*	1.89 (1.13 – 3.17)	**0.019**
Duration contact with carcass/meat					
<1h/day	18 (5%)	Reference		Reference	
< half day	34 (9%)	1.41 (0.24 – 8.09)		1.72 (0.29 – 10.30)	0.530
Whole day	316 (86%)	3.52 (1.30 – 9.52)	<0.001*	4.56 (1.51 – 14.41)	**0.011**
Livestock ownership					
Yes (vs no)	58 (16%)	0.34 (0.20 – 0.60)	0.001	0.32 (0.15 – 0.71)	**0.008**
Treatment for any chronic illness					
Yes (vs no)	91 (25%)	0.68 (0.48 – 0.97)	0.034	0.72 (0.46 – 1.13)	0.139
Animals sourced from individuals for personal consumption					
Yes (vs no)	261 (68%)	0.60 (0.41 – 0.87)	0.011	0.74 (0.51 – 1.08)	0.114

OR: odds ratio, CI: confidence interval.
*The p value was taken for the categorical variable as a whole in the univariable analysis.

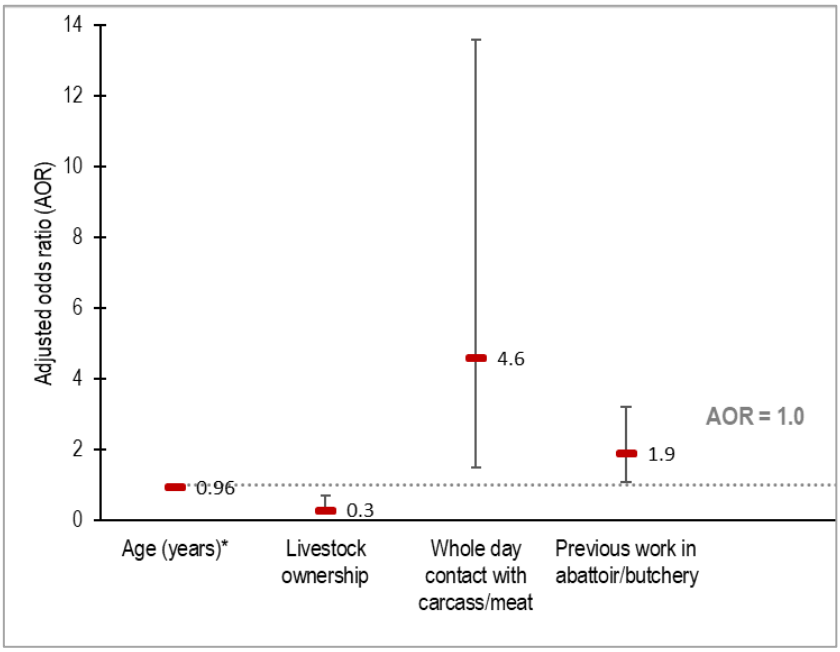

Figure 3. 1. Statistically significant factors associated with Q fever seropositivity in the multivariable analysis of the abattoir survey, South Africa, May 2018.

3.5. Sensitivity analysis

The selection of models 5 and 6 followed the same procedure as with model 4. Only the outcome variable was treated differently. The unadjusted and adjusted odds ratios for model 5 and 6 are presented here [Table 3. 9].

Model 6, in which the equivocal records were treated as negative, was more conservative and therefore contained fewer exposure variables.

Apart from the variable 'animals sourced from individuals for personal consumption', models 5 and 6 contained the same exposure variables as the final multivariable logistic regression model (model 4), and produced similar adjusted odds ratios. This shows that the individuals with equivocal results were similar to the rest of the sample population. Animals sourced from individuals for personal consumption was only absent in model 6, which was the most conservative model. The initial post-regression goodness-of-fit test result for model 5, 0.003, was poor (<0.05). However, the fit was improved to 0.053 when the abattoir size variable was removed. The post-regression goodness-of-fit test result for model 6, 0.040, was poor (<0.05).

Table 3. 9. Factors associated with Q fever seropositivity in the sensitivity analysis of the abattoir survey, South Africa, May 2018

Exposure Variable	Model 5 (equivocal specimens as positive)		Model 6 (equivocal specimens as negative)	
	OR (95% CI) [p value]	**AOR (95% CI) [p value]**	**OR (95% CI) [p value]**	**AOR (95% CI) [p value]**
Age (years)	0.96 (0.94 – 0.98) [0.002]	0.96 (0.94 – 0.98) **[0.002]**	0.96 (0.94 – 0.99) [0.009]	0.96 (0.94 – 0.98) **[0.002]**
Previous work experience				
Other	Reference	Reference	Reference	Reference
Farming	0.99 (0.44 – 2.23)	0.84 (0.42 – 1.68)	1.02 (0.52 – 2.00)	0.96 (0.53 – 1.76)
Abattoir/butcher	1.91 (0.87 – 4.20) [0.030]*	2.09 (1.12 – 3.91) **[0.022]***	1.70 (1.02 – 2.84) [0.112]*	1.81 (1.12 – 2.94) [0.070]*
Duration contact with carcass/meat				
<1h/day	Reference	Reference	Reference	Reference
< Half a day	1.15 (0.28 – 4.72)	1.32 (0.35 – 5.04)	1.45 (0.24 – 8.67)	1.86 (0.29 – 11.8)
Whole day	3.18 (1.32 – 7.62) [0.001]*	3.89 (1.56 – 9.68) **[0.006]***	3.32 (1.21 – 9.16) [0.001]*	4.43 (1.38 – 14.2) **[0.001]***
Livestock ownership	0.48 (0.32 – 0.74)	0.46 (0.25 – 0.82)	0.34 (0.19 – 0.60)	0.31 (0.15 – 0.63)
Yes (vs no)	[0.002]	**[0.012]**	[0.001]	**[0.003]**
Treatment for any chronic illness	0.61 (0.42 – 0.89)	0.68 (0.45 – 1.04)	0.74 (0.52 – 1.05)	0.78 (0.51 – 1.21)
Yes (vs no)	[0.014]	**[0.070]**	[0.084]	**[0.255]**
Animals sourced from individuals for personal consumption	0.52 (0.35 – 0.77)	0.61 (0.43 – 0.86)	0.69 (0.50 – 0.97)	
Yes (vs no)	[0.003]	[0.007]	[0.033]	

OR: unadjusted odds ratio, AOR: adjusted odds ratio, CI: confidence interval, bolded p values for factors matching model 4. The p value for categorical variables as a whole are reported.

CHAPTER 4 - Discussion & Conclusion

This chapter reflects on the study findings placing them into context of previous literature. The study limitations are explained, the research report is concluded and recommendations are given.

The chapter sub-headings are:

- 4.1 Summary of findings
- 4.2 Discussion of findings in context of the literature
- 4.3 Limitations of the study
- 4.4 Conclusion

4.1 Summary of findings

This study, a serological survey of Q fever in South African abattoir workers yielded several important findings. First, a substantial Q fever seroprevalence in the study area was estimated. Second, while controlling for the study design features, the multivariable logistic regression model identified several factors associated with Q fever seropositivity in this group. Prior experience working at an abattoir/butchery and whole day of direct contact with carcasses/meat products were positively associated with Q fever seropositivity. In contrast, increasing age and personal ownership of livestock were negatively associated. The sensitivity analysis confirmed that the individuals with equivocal Q fever test results did not differ from the individuals with conclusive test results. Notably, a history of any tick bites was not a significant exposure factor. Low rates of mask, protective eyewear and glove usage were observed for most abattoir work activities, including those with higher potential for Q fever transmission. Two exposure factors remained in the final logistic regression model but were no longer statistically significant. These were the acquisition of animals from individuals for personal consumption and treatment for any chronic illness(es).

4.2 Discussion of findings in context of the literature

Overall, about a third of abattoir workers in this group (33%) had been previously infected by *C. burnetii*. This seroprevalence is reasonably high when compared to the literature of other similar groups in whom IgG2 was also measured [79-83, 102, 105, 108]. Between country comparisons are difficult due to differences in population characteristics, sample size, study design and serological test used. However, a meta-analysis of Q fever seroprevalence in abattoir workers combined similar studies to report an overall estimate of 26%, which is slightly less than that found in this study [84]. Therefore, the seroprevalence estimate reported in our study was relatively high, at least equal to those observed in Australia at the time that the Q fever vaccination program was implemented [26].

Previous work experience at an abattoir or a butchery correlated positively with Q fever seropositivity and this is supported by the literature because working in the meat processing industry is a well-documented risk activity [24, 77, 136]. Indeed, Q fever was first recognised as an unknown fever among abattoir workers [13] and numerous outbreaks have been reported in this group since [56, 69, 79, 137]. This supports our hypothesis that personnel could be exposed to Q fever at abattoirs. In our scenario, individuals with prior work experience in the abattoir/butchery industry may have already been exposed to Q fever before

they started working at the current facility. Thus, this characteristic probably confounded the relationship between Q fever seropositivity and other exposure factors and that is why it was kept in the final model.

Longer daily duration of direct contact with carcasses or meat products emerged as a factor associated with higher rates of Q fever seropositivity. This is consistent with the literature. For example, a Dutch study found an association between Q fever seropositivity and the weekly duration of animal contact among 189 livestock veterinarians [46]. An outbreak investigation of 29 Q fever cases in France found increasing level of exposure to a slaughterhouse site to be strongly associated with seropositivity [138]. It is plausible that the more time one spends in direct contact with animal carcasses or meat products that are infected with *C. burnetii* (but appear healthy), the greater the opportunity there is for inhalation of this infectious bacterium. Although no papers that confirmed a causal relationship were found, there was good circumstantial evidence of the relationship between Q fever and animal contact. Hence, it is fair to say that the carcasses and meat products handled within abattoirs could be a source of Q fever bacteria.

Participants who personally owned livestock experienced lower Q fever seropositivity and this is contrary to the literature. In general, higher seropositivity has been observed among persons who have regular contact with livestock due to their occupations, e.g. livestock veterinarians [33, 46, 95] and livestock farmers [100]. It is reasonable to assume that the same would apply to people who have close contact with livestock due to ownership/husbandry. A study of veterinary students (n=647) reported higher Q fever seropositivity in those who lived on a ruminant farm (OR 2.7) [4] suggesting that living close to livestock is a risk factor for exposure. Although few papers included animal ownership as an independent factor for Q fever exposure, two studies of high-risk workers in western Iran found keeping of animals to be associated with increased Q fever seropositivity [81, 106]. Perhaps the assumption that personal ownership of livestock is an indication of contact with livestock in this scenario is flawed. In rural South Africa, it is common for working-age family members to migrate temporarily in search of employment at nearby towns/cities without intending to leave permanently. The older generations and children of the family usually remain at the traditional home, while the migrant family member sends remittances, food, clothing etc home [139, 140]. Any livestock owned by the family also remain at the homestead. Thus, it is possible that an individual who worked at an abattoir could have owned livestock but not have been in regular contact with them because he/she stayed at accommodation nearby the workplace. In this way, livestock ownership may have behaved more as a socioeconomic indicator than as an exposure factor. Information about where the participants lived and whether those who owned livestock lived near to the animals would have been helpful but such data were not collected.

The finding that increasing age was negatively associated with Q fever seropositivity in this analysis is contrary to what most of the literature says [48, 111-113], but the effect was small. Generally, Q fever seroprevalence increases with age because older people have had more time to become exposed than younger people. High seroprevalence has been reported among children in Africa [11] and antibody levels tend to decline over time if not stimulated. However, continuous exposure to *C. burnetii* is expected to occur in abattoir workers and this should theoretically result in persistently high antibody levels [32, 33]. Another contributing factor to this finding could be that the sensitivity of ELISA tests after acute Q fever infection decreases over time [141] leading to fewer positive specimens being detected with time. However, it is impossible to say if this effect exists and how large it would be in a population that is re-exposed to *C. burnetii*. To interpret this finding better, one would need information about age-specific human-animal contact patterns both in the family and in occupational settings. Certainly, in this scenario age group behaved as a confounder because it had a relationship with Q fever exposure and it lay outside of the causal pathway.

Two exposure variables remained in the final multivariable logistic regression model but became statistically insignificant and are briefly discussed here. The acquisition of animals from individuals for personal consumption correlated negatively with seropositivity. This could indicate a profile of animals that abattoirs could consider as low Q fever risk but there was insufficient evidence to confirm it. It is plausible that animals destined for slaughter with this characteristic could have lower risk of Q fever infection. Presumably, when a person buys live animals with the intention of consuming their meat, they tend to be more selective about the animals that they buy; choosing only those that appear fit, which come from a herd that looks healthy and a farmer whom they trust to take good care of the animals they raise. All these factors may inadvertently lead to them buying animals that are lower risk. Nonetheless, one can only speculate about this factor, as more data are needed for a definitive answer.

The other exposure variable was treatment for any chronic illness(es). This was also a negative correlate and this finding was unexpected and difficult to explain. A literature review of the relationship between acute Q fever and chronic illness yielded few papers and conflicting results [2, 16-19]. This may have been a chance finding or it may have occurred due to self-report bias. Another possible explanation could be that the medications (e.g. antibiotics) given to individuals with other chronic illnesses protected them from Q fever infection. Especially since the most common chronic condition reported was tuberculosis, which is treated with long-term antibiotics including rifampicin, an agent with anti-*Coxiella* activity. Nevertheless, there was not enough evidence for this finding to be relevant and further exploration is needed to understand if concurrent treatment for chronic illness can influence Q fever serology.

Interestingly, a history of any tick bites was not significantly associated with a previous exposure to Q fever in this sample. The literature supports the notion that tick bites are not a major route for Q fever acquisition by people [9, 10]. The idea that the *Coxiella*-like bacteria, which are found in ticks, could influence human serological responses is an interesting subject [12]. If this effect exists, it was not apparent in this dataset.

A concerning behavioural observation was that even though large proportions of sampled workers took part in abattoir activities with higher potential for Q fever transmission, the rates of mask, protective eyewear and glove usage were low. Previous literature suggests that respiratory protection masks, air filtration systems, gloves and regular disinfection of ones hands and face could protect high-risk people from Q fever infection [2, 60, 81, 99, 100, 102, 104, 119]. Thus, wearing gear to protect one's hands and face (including respiratory protection) is important for Q fever prevention in high-risk circumstances, but it is also critical to know when to take such precautions. It is must be remembered that although slaughter [117] and evisceration [102] are associated with high risk for Q fever exposure, live animals can also transmit the disease while they are kept in the holding pens [56, 80] and not just during the slaughter procedure. The health of abattoir workers can further be protected by controlling Q fever infection in the animal hosts. This can be done by the identification of infected herds and then developing a control program that is suited to each situation. This may could include culling, animal vaccinations and strict control of the movement of positive animals [9, 22, 23]. In South Africa, much work is needed towards detecting and describing the disease in herds, and the animal vaccine is not registered.

4.3 Limitations of the study

There were limitations due to the cross-sectional study design. As outcome and exposure data were collected simultaneously, our attempts to infer causality are tentative. It is estimated that IgG2 antibodies can persist for about five years [30] but Q fever antibodies may be detectable for up to 15 years [47]. In this sample, workers had been employed at the abattoir for a median of 5 years and we adjusted for prior experience working at an abattoir/butchery. Although the seropositive individuals could have been exposed anywhere in the preceding five years, since most of them were full-time employed there is a reasonable possibility that they were exposed at the abattoir. The overall seroprevalence in this group was relatively high, suggestive of exposure occurring at the abattoirs, and former work as an abattoir worker or butcher was associated with higher seroprevalence. Regardless, these findings are helpful to develop hypotheses for future studies to better understand the dynamics around Q fever exposure among abattoir workers.

Some important methodological issues arose because of the secondary nature of the project. Since the current study relied on secondary data, certain factors could not be explored further. For example, we did not have the data needed to group the abattoir workers according to the area/section in which they worked. This may have been helpful to explore if Q fever seropositivity differed for categories of abattoir workers. Similarly, the potential differences in Q fever seropositivity between workers who perform slaughtering versus evisceration versus carcass dressing could not be examined because these activities were already grouped into a single question originally. In addition, we were not able to explore if there were difference between abattoir workers who performed informal slaughter outside of working hours, where the meat is intended for personal consumption, and those who did not. This information was not collected during the primary study.

Reliance on self-report may have produced some bias, but every possible effort was made to give participants privacy when answering personal or sensitive questions. This may have been a problem in particular with the questions about treatment for chronic disease. Bias may have occurred either because of difficulty in understanding the illnesses being asked about due to language barriers or due to fears of being stigmatised. In these instances, interviewers tried to explain the questions clearly and participants submitted their answers independently on the electronic devices [personal communication with primary study epidemiologist].

It was not possible to measure differences between participants and non-participants but a large proportion, about a third, of the abattoir workers in the study area were sampled. Sampling was only limited by available time and individual refusal rate was low. There were a few exposure variables that could not be analysed because of the nature of the study design. However, those specific variables were not anticipated to emerge as major exposure factors, based on the literature review.

A potential disadvantage of any serological test is the potential for cross-reactivity with antigens from other bacteria [27]. In the case of Q fever, cross-reactivity with *Bartonella* and *Legionella* species has been recognised. However, this phenomenon was mainly reported with indirect immunofluorescence (IFA) techniques [2, 27]. This study used an ELISA assay and therefore was less likely to incur this problem. An important limitation to bear in mind is that the test we used in this study could not detect cell-mediated immunity to Q fever. It is possible that some Q fever-exposed abattoir workers might have been missed if they did not have detectable antibody titres even though they might have had cellular immunity to Q fever. Intradermal skin testing was not practically possible in this study both due to time constraints, and because Q fever vaccine is not available in the country.

Even though an acceptable level of precision and power were demonstrated for the purposes of this analysis, the sample size and area covered in the abattoir survey was not large enough for the findings to be generalisable to the entire population of abattoir workers in South Africa. The data were obtained using non-probability sampling of individuals and consequently do not constitute a representative sample. The level of threat that Q fever poses to the health of abattoir workers is directly related to the prevalence of the disease in the animals that they process. The prevalence of Q fever in animals varies greatly, depending on the animal species, animal densities, geographic area, agricultural practices and environmental factors [2, 11]. There is insufficient recent data on the prevalence of Q fever in animals in South Africa that could be used to indicate other risk areas in the country.

In this study, we used serology as a marker for exposure to Q fever but this must not be confused with clinical Q fever infection. It is possible for a person to be exposed to *C. burnetii* and produce a serological response without developing symptoms of the disease. In fact, this is the case for about 60% of people [1, 9]. We have also explained that infection in childhood is often mild or asymptomatic [2] and continuous occupational re-exposure may cause high antibody levels to persist [33]. Although a proportion of the seropositive individuals in this group most likely had clinical illness, it would be inaccurate to assume that they all experienced suffering based on our findings alone. More information, such as a follow up case-control study in this group, would assist to clarify this question.

4.4 Conclusion

A concerning level of Q fever seropositivity was detected in this group of South African abattoir workers. This observational study suggests that the workplace could be a possible source of exposure and that workers who spend the whole day in contact with raw meat could be at higher risk of Q fever exposure. From a public health perspective, this is important because there are protective measures that could be implemented to protect these individuals. The low levels of compliance with wearing protective gear for the hands and face could be improved. Occupational health medical services for abattoir workers in South Africa should include surveillance for zoonotic infectious diseases that can be contracted by persons working in the abattoir or meat processing industry.

We describe the short-term recommendations for the target population and the present study. Increased precautions should be implemented to protect abattoir workers from Q fever exposure at work. As a priority, it should be mandatory for all personnel working in direct contact with carcasses/meat products

to wear gloves, a mask and protective eyewear. Ideally, all staff who work near the live animals or their skins, or who perform slaughtering, evisceration or any cleaning up afterwards should also be obligated to wear these protective items. These measures would have the added advantage of increasing their protection from other infectious zoonotic diseases too. Abattoir staff should receive education about Q fever and training about the protective gear available to them. They should also be informed about how and where to seek medical attention should they develop any fever symptoms. This study could be strengthened if these specimens were also tested for the shorter-lived Q fever IgM antibodies against *C. burnetii* phase II. This could help to estimate the time of Q fever exposure more accurately.

In the long term, abattoir employers may consider appointing an occupational health and safety officer or strengthening the existing structures if they are already in place with this new information. This would benefit both employees, in terms of improved personal health, as well as employers, in terms of production efficiency due to reduced sick leave days if general staff health improves. Such occupational health programs can be expanded to include other important zoonotic infections associated with abattoir work for example brucellosis and leptospirosis. If further research indicates that Q fever does contribute a disease burden for people in South Africa, the disease in humans and animals should be made notifiable, as this would assist disease surveillance.

A number of knowledge gaps were identified by this study. Ideally, this work should be followed by a case-control study that would: 1) improve the understanding of clinical Q fever disease in high-risk groups in South Africa; 2) confirm/exclude risk factors associated with Q fever infection and 3) to confirm/exclude specific protective measures that are effective (e.g. types of masks to use). To understand the occupational risk better, such study should ideally be coupled with epidemiological investigation of Q fever in the surrounding livestock. There is much to learn about the *Coxiella*-like bacteria that are found in ticks [12] and more research is needed to improve the understanding of the ecology and virulence factors of the *C. burnetii* strain/s prevalent in the country. Modern molecular diagnostic techniques such as real-time PCR and genetic sequencing are excellent tools that can be used to answer such questions [12]; indeed research in this area is underway in South Africa [personal communication with Professor Marinda Oosthuizen, deputy dean of research and postgraduate studies at the Faculty of Veterinary Sciences, University of Pretoria].

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SUPPLEMENTARY SECTION

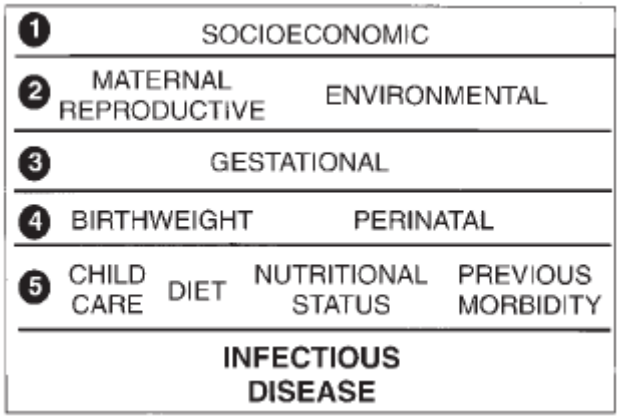
Supplementary 1. The methodology behind data manipulations made in preparation for analysis.

Original variable		**New variable**		**Reasoning**
Name	**Format**	**Name**	**Format**	
Age reported (years)	Continuous	Age calculated (years)	Continuous	Combination of age calculated from date of birth & reported age when date of birth was empty
Education level reported	Ordinal - None - Foundation - Intermediate - Senior - Further training - Higher education	Education level	Ordinal - None - Primary school - Secondary school - Higher education	Grouped based on South African norm
Tick bites	Ordinal - Never - Yes, past month - Yes, any time	Tick bites	Binary - Yes - No	Where yes any time or in the past month were combined into 'yes'
Work activities (various)	Ordinal - Never - Yes, past month - Yes, any time	Activity (various)	Binary - Yes - No	Where yes any time or in the past month were combined into 'yes'
Wearing protective clothing at work	Ordinal - Never - Sometimes - Regularly - Always	Wearing protective clothing at work	Binary - Never/sometimes - Regularly/always	'Always' was omitted from analysis due to being present for all facilities and 'never' did not have enough individuals in the group
Total staff employed (number)	Continuous	Abattoir size	Binary - Small (≤ 38 staff) - Large (> 38 staff)	Grouped by the median number of staff (facility level)
Average animals slaughtered daily (number)	Continuous	Abattoir throughput	Binary - Low (≤ 40 sheep) - High (>40 sheep)	Definition for red meat abattoirs that process > 1 species in South Africa [142]
Years established for	Continuous	Abattoir lifespan	Binary - ≤ 22 years - > 22 years	Grouped by the median number of years established (facility level)
Species of animal slaughtered (various)	Binary - Yes - No	Slaughter sheep only	Binary - Yes - No	Created a variable to compare slaughtering sheep only with slaughtering multiple species at a facility

Supplementary 2. List of exposure variables of interest for the Q fever analysis.

Questionnaire	Variable	Type	Values
	Sociodemographic		
Question 4a	Age	Continuous	Years
Question 2	Sex	Binary	Female, male
Question 3	Population group	Category	Black African, coloured, white
Question 5	Education	Category	None, primary, secondary, higher
	Health related		
Question 33	Treatment for any chronic illness	Binary	Yes, no
Question 34	Treatment for specific chronic illnesses (various)	Binary	Yes, no
Question 23	Sharp injury while working with condemned	Binary	Yes, no
Question 26	Tick bite	Binary	Yes, no
Question 30	Consumption of undercooked meat	Binary	Yes, no
	Individual level abattoir related		
Question 13	Work schedule	Binary	Part time, full time
Question 12	Period employed	Continuous	Years
Question 17	Daily duration of contact with carcass/meat	Category	<1h/day, half day, whole day
Question 16	Activities performed at work (various)	Binary	Yes, no
Question 18	Wearing protective/sanitary clothing at work	Binary	Never/sometimes, regularly/always
	Facility level abattoir related		
Question 2	Species slaughtered (various)	Binary	Yes, no
Question 2	Slaughter sheep only	Binary	Yes, no
Question 6	Source of animals (various)	Binary	Yes, no
Question 21	Personnel required to wear gloves	Binary	Yes, no
Question 21	Personnel required to wear goggles	Binary	Yes, no
Question 21	Personnel required to wear masks	Binary	Yes, no
Asked in person	Abattoir size	Binary	Small, large
Question 3	Abattoir throughput	Binary	Low, high
Question 1	Abattoir lifespan	Binary	Young, old
	Other exposures		
Question 15	Livestock ownership	Binary	Yes, no
Question 14	Previous work experience	Category	Other, farming, abattoir/butchery

Supplementary 3. Conceptual hierarchical framework for infectious diseases in developing countries [134].



Supplementary 4. Final logistic regression model equation.

The equation of the final model is given below:

$$\ln[Probability(seropositivity)] = \frac{e^{(0.634 + \beta_1(age) + \beta_2(previous\ work) + \beta_3(contact) + + \beta_4(livestock) + \beta_5(chronic) + \beta_6(source)}}{1 + e^{(0.634 + \beta_1(age) + \beta_2(previous\ work) + \beta_3(contact) + + \beta_4(livestock) + \beta_5(chronic) + \beta_6(source)}}$$

Where:	$\beta_1 = 0.96$	and age is equal to the age in years
	$\beta_2 = 0$	for previous work in "other"
	$\beta_2 = 0.86$	for previous work in farming
	$\beta_2 = 1.89$	for previous work in another abattoir/butchery
	$\beta_3 = 0$	for spending < 1h/day in contact with carcasses/meat products
	$\beta_3 = 1.72$	for spending < half the day in contact with carcasses/meat products
	$\beta_3 = 4.66$	for spending the whole day in contact with carcasses/meat products
	$\beta_4 = 0$	for not owning livestock
	$\beta_4 = 0.32$	for owning livestock
	$\beta_5 = 0$	for not being treated for any chronic illness
	$\beta_5 = 0.72$	for being treated for any chronic illness
	$\beta_5 = 0$	for not sourcing animals from individuals for personal consumption
	$\beta_5 = 0.74$	for sourcing animals from individuals for personal consumption

APPENDICES SECTION

Appendix A. Plagiarism declaration

I, Liesl De Boni (Student number: 1935335), am a student registered for the degree of MSc (Epidemiology) in the academic year 2019.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

Signature:

Date:

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Liesl De Boni

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180959

NAME (PRINCIPLE INVESTIGATOR):	Dr Liesl De Boni
DEPARTMENT:	Public Health
PROJECT TITLE:	A serological survey: Q fever among abattoir workers in Free State and Northern Cape provinces, South Africa, 2018
DATE CONSIDERED:	28/09/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Veerle Msimang
APPROVED BY:	Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	31/10/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

Appendix C. Letter of permission from the primary study

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

CENTRE FOR EMERGING ZOONOTIC AND PARASITIC
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6382 Fax: +27 (0)11 882 3741
Website: www.nicd.ac.za
Practice Number: 5200296

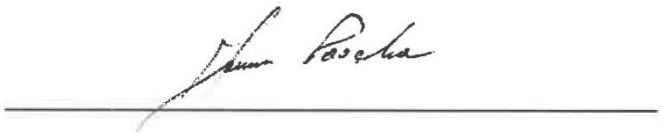
Letter of Permission for Use of Data/Samples

MEMORANDUM

DATE: 2nd August 2018

TO: Dr Liesl De Boni
MSc. Field Epidemiology student
Wits School of Public Health, Faculty of Health Sciences, the University of the Witwatersrand
SA FETP field placement at Centre for Emerging Zoonotic and Parasitic Diseases

FROM: Prof. dr hab. Janusz T. Paweska
Head Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases, National Health Laboratory Service 1 Modderfontein Road, Sandringham 2191
WHO Collaborating Centre for Reference and Research on Viral Haemorrhagic Fevers and Arboviruses
Tel: 011 386 6382 (6336), Mobile: 0829088046, E-mail: ianuszp@nicd.ac.za

SIGNATURE: 

RE: PERMISSION FOR USE OF DATA/SAMPLES

TITLE: A serological survey: Q fever among abattoir workers in Free State and Northern Cape provinces, South Africa, 2018.

- As Principal Investigator of the project "Towards a Better Understanding of Rift Valley Fever and Brucellosis Epidemiology in the Republic of South Africa (M140306)", I give student Liesl De Boni permission to analyze the data and samples collected during the abattoir serological survey of the project, expressly for the thesis project titled, "A serological survey: Q fever among abattoir workers in Free State and Northern Cape provinces, South Africa, 2018."
- No personal identifiers are present in the data to be provided. Student is given access to a separate link list of the participants.
- The data were collected with the approval of Human Research Ethics Committee - Medical, University of the Witwatersrand (M140306). The student will obtain additional ethics approval for the study.
- When publishing results forthcoming from the analysis of data, acknowledgment of EcoHealth Alliance and Defense Threat Reduction Agency and co-authorship including senior of principal investigator of project and CEZPD scientists involved with the student's study are required.

Appendix D. Facility consent form

National Institute for Communicable Diseases
Centre for Emerging, Zoonotic, Parasitic Diseases
1 Modderfontein Road, Sandringham, 2131 Tel: +27 (0)11 386 6428

EMPLOYER CONSENT

Project Title: Towards a better understanding of Rift Valley Fever and Brucellosis Epidemiology in the Republic of South Africa

Introduction

Hello my name is _____. I am from the National Institute for Communicable Diseases (NICD) / Department of Health. We are conducting a project that involves a questionnaire and the collection of blood from people residing or working in slaughter houses in the Free State and Northern Cape. We are doing this to look for exposure and risks of exposure to diseases that affect both people and livestock. These include: Rift Valley fever or other viruses (chikungunya, West Nile, Sindbis fever, Crimean-Congo haemorrhagic fever) and bacterial diseases (brucellosis, leptospirosis, Q fever, and rickettsioses).

With your permission, we will approach your employees in the abattoir/slaughter facility (up to 1015) for the study and ask if they wish to participate. Each employee will receive a consent form (attached) and can decide whether or not to participate in the study.

As outlined in the attached employee consent form, employees will have the option to volunteer to take part in the study. They will be able to choose whether or not to provide a blood sample and/or take a questionnaire. The blood sample and questionnaire will help determine prior exposure to viral and bacterial diseases. The long-term goal of the study is to better understand risk factors for viral and bacterial diseases and promote animal and public health in South Africa.

There is no cost to you or your employees to participate in the study. Participants can leave the study at any point. Participation is expected to take at most a total of 20 minutes (about 10-15 minutes for the questionnaire, and about 5 minutes to take the blood specimen). While you will not receive individual results for the participants, we will provide summary information to you about the region and it will be useful for you to look at with the animal exposure information we are also collecting.

Slaughter facility specific information: If you agree to have your slaughter facility be part of this project, we will also ask you a number of questions regarding the facility. The questions are not personal, and are focused describing the situation in your slaughter facility.

Employer permission declaration

I have read the information or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I understand what the study involves and its purpose and have voluntarily selected the following option:

Option 1

I voluntarily give my permission for my employees to take part in this study (interview) and for their blood samples to be collected for the purpose indicated above, if they also agree to participate in the study (on an individual basis). I voluntarily choose to answer questions about my slaughter facility.

Name of Employer: _____
Postal Address: _____
Email of Employer: _____
Phone Number of Employer: _____
Employer Signature: _____ Date (Day/Month/Year): _____

Option 2

I voluntarily choose to answer questions about my slaughter facility, but I do not consent for my employees to take part in this study.

Name of Employer: _____
Postal Address: _____
Email of Employer: _____
Phone Number of Employer: _____
Employer Signature: _____ Date (Day/Month/Year): _____

Option 3

I do not provide voluntarily consent for my employees to take part in this study, nor will I answer questions about my slaughter facility.

Name of Employer: _____
Postal address: _____
Employer Signature: _____ Date (Day/Month/Year): _____

Appendix E. Individual consent form

Abattoir Study 2017 – 2018 (year 4)

Informed Consent Form for Participants

Project: Understanding Rift Valley fever in the Republic of South Africa

Purpose

Several outbreaks of Rift Valley fever have occurred in South Africa causing abortions in livestock (cattle sheep and goats) and loss of particular young livestock. The disease can spread from livestock into people, while the disease is often mild in people it can cause severe disease and even death in some people. People who have direct contact with potentially infected animals are at higher risk for infection directly from fluids of- or contact with- sick animals, especially during: slaughter, handling animal parts, helping with livestock births or handling aborted material. Rift Valley fever is most commonly encountered during years in which unusually heavy rainfall and localized flooding occurs, and subsequent increase in mosquitoes that spread the virus between animals. The most recent outbreak in South Africa was in 2010-2011.

Since 2014 we have been implementing a project (approved by Witwatersrand Research Ethics Committee – M140306 and the Provincial Department of Health) to understand Rift Valley fever better and to forecast and minimize outbreaks in South Africa. We have nearly completed farm community-based surveys in 2015-2016 and 2017 to investigate past exposure of Rift Valley fever virus in animals and people within an area in the Free State and the eastern Northern Cape known to have outbreaks of the virus. We have also completed a similar survey in veterinarians. This study is also looking at weather patterns, mosquito lifecycles, soil, vegetation, livestock and wildlife to determine the contribution of each towards Rift Valley fever outbreaks.

Workers at abattoirs have been known to be infected during Rift Valley fever outbreaks, due to their potential exposure in slaughtering livestock during an outbreak. With your participation, we will conduct a survey of slaughterhouse workers (which means we collect a small blood sample from you and interview you about your about work activities) for comparison with the amount of exposure to Rift Valley fever found in farm workers during in this study. We will also compare different aspects of work at an abattoir to see if any put you at a higher risk of Rift Valley fever virus infection.

Procedures

We would invite you to complete a questionnaire and donate a blood sample of about 17 ml (< 4 teaspoons) maximum for testing of diseases you can get from animals: Rift Valley fever, Congo fever, brucellosis and possibly leptospirosis, Q fever and rickettsioses, and chikungunya, West Nile, and Sindbis fever .The research questions are mainly related to the work you do with animals and the illness you may have experienced recently or previously in association with Rift Valley fever, Congo fever, brucellosis or one of the other diseases mentioned above that you can get from animals. It is anticipated that the questionnaire and blood sampling will take approximately half an hour of your time. We would like you to volunteer participating in this part of the study.

Benefits and risks

The likely benefits to you are minimal but the overall impact of participation will be very significant to our community and South Africa because the information we collect from you and other participants will be combined to develop understand of Rift Valley fever or Congo fever or other diseases mentioned above that you can get from animals and find ways to minimize them. You may feel a bit of discomfort during the drawing of the blood and/or have small temporary bruising at the site of blood draw for few days. You can skip questions you feel uncomfortable with answering them. You will not be identified with the information you give because the research is confidential. A number will be assigned to your survey questions and blood sample and not linked to your identifying information you complete here.

Respondent agreement

The research study has been explained to me. I voluntarily participate. I had an opportunity for my questions to be answered. I know that I may stop my participation in the research at any time. I understand that if I have questions about this research and my participation, I may contact the epidemiologist at the Centre for Emerging and Zoonotic Diseases, National Institute for Communicable Diseases (011 3866428 or email veerlem@nicd.ac.za).

_ _ / _ _ /_ _ _ _

Participant's Signature:	Date	Name *(write complete and clearly)*
Participant's Tel. No.	Cell No.	Email
Participant's study No.	Farm study No.	Address *(write complete and clearly)*

Appendix F. Facility questionnaire

Gray text is not visible to the person taking the survey, but in place to assist with the flow of the survey in paper format (how it will be given on the ODK app on a tablet).

To be filled out by survey administrator:

Admin 1. Scan the barcode for the associated record.

Admin 1a. Enter the barcode manually.
If scanning the barcode is not possible:

Admin 2. Slaughter facility identifier:

Admin 2a. Slaughter facility name:

Admin 3. Date of interview:

Admin 4. Collect the current GPS coordinates.

Admin 4a. Enter the latitude and longitude manually using the GPS. If automatic GPS coordinate does not work then:

Latitude (Degrees) _____
Latitude (Minutes) _____
Latitude (Seconds) _____
Longitude (Degrees) _____
Longitude (Minutes) _____
Longitude (Seconds) _____

Admin 5. Filled in by: _____

The remainder of the survey should be filled out by the participant.

1. When was this slaughter facility established? *Please use numbers for month and year.* ____Month ___Year

2. Which animals are slaughtered at this facility?
☐ Cattle
☐ Sheep
☐ Goats
☐ Pigs
☐ Poultry
☐ Other
If selected "Other" in Q2: Please specify what other animals are slaughtered at this facility: _____

3. What is the average number of animals slaughtered on most days? _____
4. How many days of the year does this slaughter facility operate? _____
5. What type of employees do you have: *Select all that apply.*
☐ Permanent ☐ Temporary
5a. If selected permanent: How many permanent employees work at the slaughter facility? _____
5b. If selected temporary: How many temporary employees work at the slaughter facility? _____

6. What is the source of animals for slaughter? *Select all that apply.*
☐ Private farms
☐ Community farms
☐ Imported
☐ Auction
☐ From an individual for their personal consumption
☐ Other
If selected "Other" in Q6: Specify other source: _____
If selected "Imported" in Q6: Please name countries from which the animals are imported: _____ If selected "Private farms" in Q6: Please indicate number of private farms: _____
If selected "Community farms" in Q6: Please indicate number of community farms: _____

7. Where are meat/carcasses or product sold? *Select all that apply.*

☐ At slaughterhouse
☐ Butcheries
☐ Supermarket
☐ Exported
☐ Other

If selected "Other" in Q7: Please indicate other locations your meat/carcasses are sold: _____

If selected "Exported" in Q7: Please name countries to which your meat/carcasses are exported: _____

8. Is the slaughter house equipped with:

A lairage (where animals are kept before slaughter):	☐ Yes	☐ No
Water/feeding troughs for animals while waiting	☐ Yes	☐ No
A site for live animal inspection:	☐ Yes	☐ No
A restraint facility where the animal is stunned:	☐ Yes	☐ No
A gutter for blood drainage present:	☐ Yes	☐ No
A refrigerated meat storage facility:	☐ Yes	☐ No
A waste disposal facility	☐ Yes	☐ No
An area to disinfect animal transport vehicles:	☐ Yes	☐ No
If selected "Poultry" in Q2		
Scalding facilities:	☐ Yes	☐ No
De-feathering facilities:	☐ Yes	☐ No

9. Do you use insect control at the slaughter facility? ☐ Yes ☐ No

If selected "Yes" in Q9: 9a. Do you use insect control in the meat storage area? ☐ Yes ☐ No

10. Do you use rodent control at the slaughter facility? ☐ Yes ☐ No

If selected "Yes" in Q10: 10a. Do you use rodent control in the meat storage area? ☐ Yes ☐ No

11. Is the water used to clean the knives used for slaughtering and cutting heated to at least 55ºC at the nozzle? ☐ Yes ☐ No

12. Is the water quality tested? ☐ Yes ☐ No

13. Is there a back-up system to maintain the temperature for preservation of meat in the refrigerator? ☐ Yes ☐ No

13a. Is there a back-up system to maintain the temperature for preservation of meat in the freezer? ☐ Yes ☐ No

14. Is the slaughtering/cutting room ventilated to prevent the accumulation of harmful gas, vapour and bad smells? ☐ Yes ☐ No

15. Are the livestock products handled and moved through the facility without contact with the floor and walls? ☐ Yes ☐ No

16. Does the slaughter facility provide a changing room for employees? ☐ Yes ☐ No

17. Are toilets present in the slaughter facility? ☐ Yes ☐ No

17a. Are toilets separated from the slaughtering and meat rooms? ☐ Yes ☐ No

17b. Are employees required to wash their hands after using the toilet? ☐ Yes ☐ No

17c. Is there insect and mice control for the toilets? ☐ Yes ☐ No

18. Is the slaughterhouse required to follow the HACCP (Hazard Analysis and Critical Control Points) administration standard? ☐ Yes ☐ No

18a. Does the slaughterhouse adhere to the HACCP administration standard? ☐ Yes ☐ No

18b. When was this slaughterhouse was approved by the HACCP? ____ Day ____ Month _____ Year

19. Are there written standard operating procedures to ensure sanitation at the slaughter facility? ☐ Yes ☐ No

19a. Are regular sanitation assessments conducted in accordance with the standard operating procedures in the slaughter facility? ☐ Yes ☐ No

19b. Does the slaughter facility keep records of the sanitation assessments for more than 6 months? ☐ Yes ☐ No

20. Is the slaughtering room and associated equipment cleaned before slaughtering commences? ☐ Yes ☐ No

If selected "Yes" in Q20: Please indicate whether before:

☐ Each day
☐ Each shift

20a. Is the slaughtering room and associated equipment cleaned after slaughtering is completed ☐ Yes ☐ No

If selected "Yes" in Q20a: Please indicate whether after:

☐ Each day
☐ Each shift

21. Are personnel at the slaughter facility required to wear: *Select all that apply.*

☐ Plastic aprons
☐ Overalls
☐ Gumboots
☐ Shoe covers
☐ Sanitary cap
☐ Gloves
☐ Mask
☐ Goggles/face shield
☐ Other

If selected "Other" in Q21: Please indicate other protective equipment personnel are required to wear:

_____

22. Do workers disinfect slaughtering knives and utensils with water of at least 55ºC at the nozzle to prevent contamination of carcass during slaughtering? ☐ Yes ☐ No

If selected "Yes" in Q22: Please indicate how often:

☐ Always compliant (100%)
☐ Regularly compliant (100-75%)
☐ Sometimes compliant (50-25%
☐ Rarely compliant (0-25%)

23. How frequently are workers formally reminded (through a presentation, during a meeting, visual signs or checklist posted at facility) of the standard operating procedures for sanitation used at this slaughter facility?

☐ Yearly
☐ Monthly
☐ Daily
☐ Never
☐ Other

If selected "Other" in Q23: Please indicate the frequency of formal sanitation procedure reminders: _____

24. Are meat/carcass inspections conducted at the slaughter facility? ☐ Yes ☐ No

If selected "Yes" in Q24: How often?

☐ Daily
☐ Weekly
☐ Monthly
☐ Quarterly
☐ Yearly

25. What type of inspectors work at the slaughter facility? *Select all that apply.*

☐ State veterinarians
☐ Private veterinarians
☐ Animal health technicians
☐ Other

If selected "Other" in Q25: Please indicate the profession of the other inspectors:

_____

25a. If selected "State veterinarians" in Q25: How many state veterinarians work at the slaughter facility currently? _____

25b. If selected "Private veterinarians" in Q25: How many private veterinarians work at the slaughter facility currently? _____

25c. If selected "Animal health technicians" in Q25: How many animal health technicians work at the slaughter facility currently? _____

25d. If selected "Other" in Q25: How many other inspectors work at the slaughter facility currently? _____

26. Which meat/carcass regulatory tests are conducted? *Select all that apply.*

- ☐ Antibiotic residue tests on kidneys conducted by random sampling
- ☐ Environmental microbial tests (e.g. bacterial culture for pathogens such as *Staphylococcus aureus, Salmonella* spp.)
- ☐ Carcass microbial tests (e.g. bacterial culture for pathogens such as *Staphylococcus aureus, Salmonella* spp.)
- ☐ pH determination of meat (24 hrs post slaughter)
- ☐ Bile pigment determination of meat
- ☐ Organo-leptic testing of meat (24 hrs post slaughter)
- ☐ Testing for viable tapeworm
- ☐ Examination for tapeworm cysts by incision of muscles
- ☐ Examination for lymphnode lesions by incision of lymphnodes
- ☐ Testing the moisture content of the bone Marrow
- ☐ Agar sausage technique testing Other:

If selected "Other" in Q26: What other tests are used to ensure quality at the slaughter facility currently?

_____

27. How frequently are tests of meat quality conducted?

- ☐ Yearly
- ☐ Monthly
- ☐ Daily
- ☐ Never
- ☐ Other

If selected "Other" in Q27a: Please indicate the frequency of meat quality tests: _____

27a. Are the results of the meat quality tests kept for more than 6 months? ☐ Yes ☐ No

28. How many carcasses were condemned in the past year? _____

28a. If selected answer in Q28 is >0: What were the reasons for condemnation?

_____

29. How many carcasses were contaminated in the past year? _____

29a. If selected answer in Q29 is >0: What were the reasons for contamination?

_____

30. What are the protocols for treatment of apparently sick animals?

_____

31. What are the protocols for reporting on water/sanitation quality by the facility to state veterinary services?

_____

32. What are the protocols for reporting on condemned meat by the facility to state veterinary services?

_____

Thank you for participating in the survey

Appendix G. Individual questionnaire

The question number indicates BOTH the facility owners/managers AND the workers' answers to the question. "1" indicates that only the facility owners/managers were required to answer the question. Gray text is not visible to the person taking the survey, but in place to assist with the flow of the survey in paper format (vs how it will be given on the ODK app on a tablet).

To be filled out by survey administrator:

Admin 1. Scan the barcode for the associated record.

Admin 1a. Enter the barcode manually.
If scanning the barcode is not possible:

Admin 2. Slaughter facility identifier:

Admin 2a. Slaughter facility name:

Admin 3. Date of interview:

Admin 4. Collect the current GPS coordinates.

Admin 4a. Enter the latitude and longitude manually using the GPS. If automatic GPS coordinate does not work then:

Latitude (Degrees) _____
Latitude (Minutes) _____
Latitude (Seconds) _____
Longitude (Degrees) _____
Longitude (Minutes) _____
Longitude (Seconds) _____

Admin 5. Filled in by: _____

Admin 6. Interview conducted? ☐ Yes ☐ No

Admin 7. Blood sample collected? ☐ Yes ☐ No

Admin 8. Is the participant the owner/manager of the slaughter facility? ☐ Yes ☐ No

The remainder of the survey should be filled out by the participant.

2. Gender: ☐ Male ☐ Female

3. Race:
☐ Black African
☐ Coloured
☐ White
☐ Indian
☐ Asian

4a. *Answer at least one:*
How old are you? _____ years
4b. Date of birth: *Please use numbers to indicate month.*
_____ Day
_____ Month
_____ Year

5. What is your level of education?

☐ No school
☐ Foundation (Gr1-3)
☐ Intermediate (Gr4-6)
☐ Senior (Gr7-9)
☐ Further Education and Training/FET (Gr10-12) Higher
☐ Education/HE

6. Have you heard about animal or human disease outbreaks in the province? ☐ Yes ☐ No
(An outbreak is when a number of animals and/or humans become ill with the same symptoms around the same time.)

7. Have you heard of Rift Valley fever? ☐ Yes ☐ No

7a. If selected "Yes" to Q7: Please indicate the source of this information: *Select all that apply.*
☐ Infected animals that were brought to my slaughter house
☐ State Veterinary Services
☐ Radio
☐ Television
☐ Internet
☐ Neighbour or friend's facility or farm was infected/affected
☐ Private Veterinarian
☐ Seminar/information session/presentation
☐ Other
If other: Please specify other information source: _____

7b. If selected "Yes" to Q7. Do you know how people catch Rift Valley fever? ☐ Yes ☐ No

7c. If selected "Yes" to Q7b. People can catch Rift Valley fever from …*Select all that apply.*
☐ Drinking raw milk
☐ Eating raw/uncooked meat
☐ Physical contact with (touching) sick animals
☐ Physical contact with (touching) dead/aborted animals/tissues
☐ Physical contact with (touching) healthy animals
☐ Physical contact with (touching) carcasses/meat
☐ Physical contact with (touching) condemned carcasses/meat
☐ Bite from an animal
☐ Bite from a mosquito
☐ Bite from a tick
☐ Physical contact with (touching) sick people
☐ None of the above

8. Have you heard of Congo fever (Crimean-Congo haemorrhagic fever)? ☐ Yes ☐ No

8a. If selected "Yes" in Q8. Please indicate the source of this information: *Select all that apply*.
☐ Infected animals that were brought to my slaughter house
☐ State Veterinary Services
☐ Radio
☐ Television
☐ Internet
☐ Neighbour or friend/'s facility or farm was infected/affected
☐ Private Veterinarian
☐ Seminar/information session/presentation
☐ Other
If other: Please specify other information source: _____

8b. If selected "Yes" in Q8. Do you know how people catch Congo fever? ☐ Yes ☐ No

8c. If selected "Yes" to Q8b. People can catch Congo fever from …*Select all that apply.*

- ☐ Drinking raw milk
- ☐ Eating raw/uncooked meat
- ☐ Physical contact with (touching) sick animals
- ☐ Physical contact with (touching) dead/aborted animals/tissues
- ☐ Physical contact with (touching) healthy animals
- ☐ Physical contact with (touching) carcasses/meat
- ☐ Physical contact with (touching) condemned carcasses/meat
- ☐ Bite from an animal
- ☐ Bite from a mosquito
- ☐ Bite from a tick
- ☐ Physical contact with (touching) sick people
- ☐ None of the above

9. Have you heard of brucellosis (undulant fever/Malta fever)? ☐ Yes ☐ No

9a. If selected "Yes" in Q9. Please indicate the source of this information: *Select all that apply.*

- ☐ Infected animals that were brought to my slaughter house
- ☐ State Veterinary Services
- ☐ Radio
- ☐ Television
- ☐ Internet
- ☐ Neighbour or friend/'s facility or farm was infected/affected
- ☐ Private Veterinarian
- ☐ Seminar/information session/presentation
- ☐ Other

If other: Please specify other information source: _____

9b. If selected "Yes" in Q9. Do you know how people catch brucellosis? ☐ Yes ☐ No

9c. If selected "Yes" to Q9b. People can catch brucellosis from …*Select all that apply.*

- ☐ Drinking raw milk
- ☐ Eating raw/uncooked meat
- ☐ Physical contact with (touching) sick animals
- ☐ Physical contact with (touching) dead/aborted animals/tissues
- ☐ Physical contact with (touching) healthy animals
- ☐ Physical contact with (touching) carcasses/meat
- ☐ Physical contact with (touching) condemned carcasses/meat
- ☐ Bite from an animal
- ☐ Bite from a mosquito
- ☐ Bite from a tick
- ☐ Physical contact with (touching) sick people
- ☐ None of the above

10. Can people catch any disease in any of the following ways?

Drinking raw milk	☐ Yes	☐ No	☐ I don't know
Eating raw/uncooked meat	☐ Yes	☐ No	☐ I don't know
Physical contact with (touching) sick animals	☐ Yes	☐ No	☐ I don't know
Physical contact with (touching) dead animals	☐ Yes	☐ No	☐ I don't know
Physical contact with (touching) healthy animals	☐ Yes	☐ No	☐ I don't know
Physical contact with (touching) carcasses/meat	☐ Yes	☐ No	☐ I don't know
Physical contact with (touching) condemned carcasses/meat	☐ Yes	☐ No	☐ I don't know
Bite from an animal	☐ Yes	☐ No	☐ I don't know
Bite from a mosquito	☐ Yes	☐ No	☐ I don't know
Bite from a tick	☐ Yes	☐ No	☐ I don't know

11. What type of work?

☐ Abattoir owner
☐ Abattoir manager
☐ Abattoir worker
☐ Abattoir clean
☐ Mechanical worker
☐ Veterinarian
☐ Veterinary Specialist
☐ Animal Health Technician
☐ Veterinary Technologist
☐ Other

If selected "Other" in Q11: Specify other type of work:

_____

11a. If selected "Veterinarian" or "Veterinary Specialist" in Q11 Are you a registered vet specialist?

☐ Yes ☐ No

If selected "Yes" in Q11a. Please select specialty: *Select all that apply.*

☐ Veterinary Public Health
☐ Small animal specialty
☐ Medicine: Small Stock or Bovids
☐ Medicine: Other
☐ Reproduction (Genesiology, theriogenology): Small Stock or Bovids
☐ Reproduction (Genesiology, theriogenology): Other
☐ Specialist Practitioner: Small Stock, Game or Bovids
☐ Specialist Practitioner: Other
☐ Other

If selected "Other": Specify other specialization: _____

12. How long have you worked at this slaughter facility?
Answer to one of the questions is required.
Answer either in years or months. Years: _____ Months: _____

12a. If answer is less than 7 years: Were you employed at an abattoir in 2010-2011? ☐ Yes ☐ No

12b. If yes: Where were you employed during 2010-2011?
Name of abattoir: _____ City:_____

13. What is your work schedule at this slaughter facility? *Check all that apply.*

☐ Full-time
☐ Part-time
If "part-time" is selected: Please indicate the number of hours per week you work: _____
☐ Shifts
If "shifts" is selected: Please indicate which shifts you work:
☐ Morning ☐ Afternoon ☐ Day ☐ Night ☐ Weekend
Please indicate the number of hours per shift you work: _____
Please indicate the number of shifts per month you work: _____
☐ Seasonal
If "seasonal" is selected: Please indicate the number of months per year you work: _____

If selected "Veterinarian" or "Veterinary Specialist" or "Animal Health Technician" or "Veterinary Technologist" in Q11 and Part-time in Q11d. respond to 13a-d.

13a. What percentage of your time working is spent …? *Please answer in percent of your hours per week.*
At any slaughter facilities: _____
At a veterinary clinic: _____
Making veterinary farm calls: _____

13b. If time at slaughter facilities is >0: Approximately how many slaughter facilities do you work at during an average week? _____

13c. If time on farm calls is >0: Approximately how many farms do you visit during an average week? _____

13d. If time on farm calls is >0: How large is your client area? *Please indicate approximate radius of client area in km.* _____

14. Where have you worked prior to your current job? *Select all that apply.*

☐ Other slaughter facility/butchery
☐ Private livestock farm (including feedlot, dairy or other type)
☐ Community farm
☐ Auction
☐ Game/wildlife farm
☐ Agricultural show
☐ Other

If selected "Other" in Q14: Please specify other work setting: _____

15. Do you personally own any livestock? ☐Yes ☐ No

15a. If yes: Please indicate what species of livestock you own:

☐ Cattle
☐ Sheep
☐ Goats
☐ Pigs
☐ Poultry
☐ Other: _____

If selected "Other" in Q15: Please specify other animals you own: _____

16. Are you involved in any of the following activities at the slaughter facility? *Please scroll down to view all choices.*

Receiving and/or holding of livestock: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐ Never
Slaughter, evisceration and/or carcass dressing: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Packaging meat product(s): ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Freezing finished carcasses and/or packaged product: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Processing hides: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐ Never
Treating wastewater: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Transporting processed material: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Cleaning equipment: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Cleaning slaughtering areas: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
General cleaning outside of the slaughtering areas: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Collecting microbial samples: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Carcass/meat inspection: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Clinical exam of a live animal: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never
Other close contact with animals or their products: ☐Yes, IN THE PAST MONTH ☐Yes, AT ANY TIME IN THE PAST ☐Never

If other "Yes, IN THE PAST MONTH": Please explain what other contact with animals or their products you have had IN THE PAST MONTH:

_____

If other "Yes, AT ANY TIME IN THE PAST": Please explain what other contact with animals or their products you have had AT ANY TIME IN THE PAST:

_____

17. On a typical day, how long are you in direct physical contact with carcasses/meat?

☐ < 1 hour
☐ < 1/2 day
☐ Whole day

18. Do you wear protective or sanitary clothing while working in the slaughter facility? (e.g. apron, overall, boots, shoe covers, mask, gloves, cap, etc.)

☐ Always (100% of the time)
☐ Regularly (100-75%% of the time)
☐ Sometimes (50-25% of the time)
☐ Never or rarely (0-25% of the time)

19. What type of protective clothing do you wear while in performing the following activities:

Bring forward only the activities indicated in Q16.

Receiving and/or holding of livestock: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Slaughter, evisceration and/or carcass dressing: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Packaging meat product(s): ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Freezing finished carcasses and/or packaged products: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Processing hides: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Treating wastewater: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Transporting processed material: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Cleaning equipment: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Cleaning slaughtering areas: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

General cleaning outside of the slaughtering areas: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Sanitary shoes ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Collecting microbial samples: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Carcass/meat inspection: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Clinical exam of a live animal: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

Other close contact with animals: ☐ Plastic apron ☐ Overall ☐ Gumboots ☐ Shoe covers ☐ Cap ☐ Gloves ☐ Mask ☐ Goggles/faceshield ☐ None

20. When do you wash your hands while working? *Select all that apply.*

☐ Before touching animals or carcasses
☐ Always between touching animals or carcasses
☐ After you are finished working or before going home
☐ Before eating

20a. If before touching animals: How often do you wash your hands before touching animals or carcasses?

☐ Always (100% of the time)
☐ Regularly (100-75%% of the time)
☐ Sometimes (50-25% of the time)
☐ Never or rarely (0-25% of the time)

20b. If after finished: How often do you wash your hands after you are finished working or before going home?

☐ Always (100% of the time)
☐ Regularly (100-75%% of the time)
☐ Sometimes (50-25% of the time)
☐ Never or rarely (0-25% of the time)

20c. If before eating: How often do you wash your hands before eating?

☐ Always (100% of the time)
☐ Regularly (100-75%% of the time)
☐ Sometimes (50-25% of the time)
☐ Never or rarely (0-25% of the time)

20d. If selected "Sometimes" or "Always" in Q18: What do you usually wash and dry your hands with? *Select all that apply.*

☐ Water
☐ Soap
☐ Disinfectant hand wash
☐ Disposable paper towel
☐ Hand towel
☐ Wipe on your clothes
☐ Air dry

20e. If "soap" is selected: Please indicate the name of the soap you use: _____

20f. If "disinfectant hand wash" is selected: Please indicate the name of the disinfectant hand wash you use: _____

20g. If selected "Water" in Q20d: What is the source of the hand washing water? *Select all that apply.*

☐ Tap in the bathroom
☐ From a hose
☐ From a bucket
☐ Other

If other Please specify other sources of water you use to wash your hands: _____ (Not required)

21. To your knowledge, have you been working with suspected disease, confirmed diseases, or condemned animals/carcasses IN THE PAST MONTH?

☐ Yes ☐ No

21a. If yes: Which animals did you work with that were diseased or condemned? *Select all that apply.*

☐ Cattle
☐ Sheep
☐ Goats
☐ Pigs
☐ Poultry
☐ Other animals

21b. If selected "Other animals": What other species of sick animals/carcasses did you work with IN THE PAST MONTH? _____

21c. For the past month, please describe the infectious disease(s) (suspected or confirmed) that the animal(s) had, the reason for condemnation of carcasses and what type of contact you had. *Please indicate the disease or clinical signs and species for all diseases or condemnations you have seen in the past month (e.g. Slaughtered cattle with tuberculosis)*

_____
_____
_____

21d. To your knowledge, have you been working with suspected disease, confirmed diseases, or condemned animals/carcasses AT ANY TIME IN THE PAST?

☐ Yes ☐ No

21e. If yes: Which animals did you work with that were diseased or condemned? *Select all that apply.*

☐ Cattle
☐ Sheep
☐ Goats
☐ Pigs
☐ Poultry
☐ Other animals

21f. If selected "Other animals": What other species of sick animals/carcasses did you work WITH ANY TIME IN THE PAST? _____

21h. For any time in the past, please describe the infectious disease(s) (suspected or confirmed) that the animal(s) had, the reason for condemnation of carcasses and what type of contact you had. *Please indicate the disease or clinical signs and species for all diseases or condemnations you have seen at any time in the past (e.g. Slaughtered cattle with tuberculosis)*

_____
_____

22. To your knowledge, have you ever worked with animals/carcasses with suspected or confirmed Rift Valley fever AT ANY TIME IN THE PAST? ☐ Yes ☐ No

22a. If yes: Which animals did you work with that had Rift Valley fever? *Select all that apply.*

☐ Cattle
☐ Sheep
☐ Goats
☐ Pigs
☐ Poultry
☐ Other animals

22b. If selected "Other animals": What other species of animals did you work with IN THE PAST that had Rift Valley fever? _____

23. Have you suffered an injury from a sharp object while working with diseased animals or condemned carcasses?

☐ Yes ☐ No

23a. If "yes" to 23. Please explain the circumstances of the injury and describe the sharp object: *For example, "I cut my finger with a knife while working on the carcass"*

_____

24. Do you live within 20 km of a lake/dam/pan/vlei/river or open water source (other than water pump)?

☐ Yes ☐ No

24a. Do you live within 1 km of a lake/dam/pan/vlei/river or open water source (other than water pump)?

☐ Yes ☐ No

24b. If selected "Yes": Please indicate the approximate distance between the lake/dam/pan/vlei/river or open water source and your home:

In meters _____ OR in minutes walking _____

25. Have you had any mosquito bites in the past month? ☐ Yes ☐ No ☐ I don't know

25a. Do you get mosquito bites easily? ☐ Yes ☐ No

25b. Do you use anything to prevent mosquitoes biting you? ☐ Yes ☐ No

25c. If selected Yes: What do you use to prevent mosquito bites? *Select all that apply.*

☐ Mosquito nets
☐ Screened windows
☐ Repellent/spray
☐ Other

If selected "Other": Please list other ways you prevent mosquitoes from biting you:

_____

26. Have you had a tick bite? ☐ Yes, in the past month ☐ Yes, at any time in the past ☐ Never

27. Have you taken ticks from hooved animals and squashed them between your fingers?

☐ Yes, in the past month ☐ Yes, at any time in the past ☐ Never

28. Have you ever eaten meat from a hooved animal found dead? ☐ Yes ☐ No

28a. If selected "Yes" in Q28. Specify animal: _____

28b. If selected "Yes" in Q28. Was this animal domestic or wild? ☐ Domestic ☐ Wild

29. Do you drink milk? ☐ Yes ☐ No

29a. If selected "Yes" in Q29. Is the milk: Always
☐ Raw
☐ Always boiled/pasteurized at home
☐ From a shop
☐ On occasion raw, other times boiled/pasteurized

29b. If selected "From a shop" in Q29a. Please describe the packaging it comes in or indicate what the brand of the milk is if known: *For example, Parmalat in a carton.*

_____

30. Do you eat rare or not fully cooked meat? *Do not include meat from birds or fish.* ☐ Yes ☐ No

30a. If selected "Yes" in Q30. Is the meat: Always
☐ Rare/medium rare
☐ On occasion rare, other times fully cooked

31. Have you experienced any of the following symptoms in the...

Fever	☐ Last two weeks	☐ Last year	☐ Never
Sore joints (more than one, not from work/sport)	☐ Last two weeks	☐ Last year	☐ Never
Headache	☐ Last two weeks	☐ Last year	☐ Never
Tiredness (not because you haven't slept)	☐ Last two weeks	☐ Last year	☐ Never
Feeling generally unwell	☐ Last two weeks	☐ Last year	☐ Never
Neck stiffness (not from injury)	☐ Last two weeks	☐ Last year	☐ Never
Loss of appetite	☐ Last two weeks	☐ Last year	☐ Never
Nausea, vomiting, stomach cramps	☐ Last two weeks	☐ Last year	☐ Never
Diarrhea	☐ Last two weeks	☐ Last year	☐ Never
Sore, red eyes, sensitive to light	☐ Last two weeks	☐ Last year	☐ Never
Blurred or loss of vision	☐ Last two weeks	☐ Last year	☐ Never
Confusion/hallucinations	☐ Last two weeks	☐ Last year	☐ Never
Skin rash	☐ Last two weeks	☐ Last year	☐ Never
Bleeding (not due to any injury)	☐ Last two weeks	☐ Last year	☐ Never
Profuse sweats	☐ Last two weeks	☐ Last year	☐ Never
Weight loss (unusual)	☐ Last two weeks	☐ Last year	☐ Never

Please describe the skin rash you had in the last two weeks: _____ If selected "Last two weeks" for "Skin rash" in Q28.

Please describe the bleeding you had in the last two weeks: _____ If selected "Last two weeks" for "Bleeding" in Q28.

Please describe the skin rash you had in the last year: _____ If selected "Last year" for "Skin rash" in Q28.

Please describe the bleeding you had in the last year: _____ If selected "Last year" for "Bleeding" in Q28.

32. If selected "Last two weeks" or "Last year" for any symptom in Q31. Did you go to a doctor/clinic/hospital when you were ill with these symptoms? ☐ Yes ☐ No

If selected "Yes" in Q32.
Please name the health facility and location: _____
What was the diagnosis? _____

33. Are you currently on any long-term medication (for longer than one month)? Yes ☐ No ☐

If selected "Yes" in 33: Please specify the medication(s): _____

34. Please indicate any chronic conditions you are being treated for: *Select all that apply.*

- ☐ Tuberculosis (TB)
- ☐ Asthma
- ☐ Heart disease
- ☐ Liver disease
- ☐ Kidney disease
- ☐ Obesity
- ☐ Diabetes
- ☐ Immunosuppression (e.g. HIV, on immunosuppressive medication, malignancy)
- ☐ Rheumatoid arthritis (debilitating inflammatory joints or deformities)
- ☐ Other
- ☐ None

If other: Please describe any health problems you have had in the past year: _____

35. For females only: Have you ever had a miscarriage? ☐ Yes ☐ No

36. Have you ever been diagnosed with Rift Valley fever? ☐ Yes ☐ No
37. Have you ever been diagnosed with Congo fever? ☐ Yes ☐ No
38. Have you ever been diagnosed with brucellosis? ☐ Yes ☐ No

If selected "Yes" in 36.
Please provide year(s) of diagnoses: _____
Please indicate the laboratory that diagnosed you, if known: _____
If selected "Yes" in 37.
Please provide year(s) of diagnoses: _____
Please indicate the laboratory that diagnosed you, if known: _____
If selected "Yes" in 38.
Please provide year(s) of diagnoses: _____
Please indicate the laboratory that diagnosed you, if known: _____

39. Have you ever been hospitalized in the past 10 years? ☐ Yes ☐ No
If yes: Please indicate the reason why you were hospitalized:
_____
If yes: Please indicate what year you were hospitalized:
_____

40. Have you been taking care of or been in close contact with (touching) a sick person in the past month?
☐ Yes ☐ No
If "Yes" in 40: Please specify the illness that person had: _____

You have successfully completed the survey! Thank you for your participation! Please hand your device back to the administrator.

Attention survey administrator. Please add any notes here: _____

Thank you for participating in the survey