A case control study of risk factors for bovine brucellosis in the Eastern Cape Province, South Africa

By

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Submitted in partial fulfilment of the requirements for the degree of Master of Science (Animal/Human/Ecosystem Health)

in the

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Declaration

I hereby declare that this dissertation, which I hereby submit for the Master of Science degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, to be my own work and has not been previously submitted by me for degree purposes at another tertiary institution.

George Tapiwa Sandengu

October 2018

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List of Abbreviations

CFT	Complement fixation test
CI	Confidence Interval
DAFF	Depart of Agriculture, Forestry and Fisheries
ELIZA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation of the United Nations
OIE	World Organisation for Animal Health
OR	Odds ratio
WHO	World Health Organisation

Dissertation Summary

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Degree: **MSc (Animal/Human/Ecosystem Health)**
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Bovine brucellosis is a worldwide, zoonotic infection caused by *Brucella* species bacteria and characterised by abortions and retained placentae in cows and, to a lesser extent, orchitis in bulls. The disease is a zoonotic risk (causing undulant fever, Mediterranean fever or Malta fever in humans) for those working with breeding cattle and threatens both food security and food safety. Accordingly, control and ultimately eradication of the disease is a goal of most countries where it occurs in order to enhance animal health and protect human health.

The aim of this study was to assess the herd level risk factors associated with occurrence of brucellosis in the Eastern Cape, South Africa, in order to assist the veterinary authorities to implement and/or enhance strategies that can control the disease at farm level. The study is part of a multiple-location study in different provinces of South Africa to investigate risk factors where case numbers are limited locally but where the power of the study is increased, when combined with the other concomitant studies.

A case control study design was used. Case herds were defined as those with culture-positive herds or more than two complement fixation test (CFT) - positive reactors, in the absence of adult Strain 19 vaccination, between 2013 and 2017. Control herds were defined as those that tested negative within six months of infection being detected in case herds and which had no history of brucellosis. A total of 77 farms were recruited for the study, comprising 30 cases and 47 controls. A pre-trialled questionnaire was used to conduct interviews on case and control farms by trained animal health officials. Assessed

risk factors included herd characteristics, cattle movements, potential brucellosis contacts, presence of wildlife and management/employee knowledge. Data were transferred to a Microsoft Access 2013 database and analysed in Excel 2013 and SPSS (IBM, Version 25). A univariate analysis was undertaken to examine the association between case-control status and potential risk factors. Significant risk factors at that stage included abortions in the herd, *Brucella* positive neighbours, use of artificial insemination with or without a bull, the proportion of cows/heifers greater than 0.64, the farming status of the herd (i.e. being commercial) and herd type (dairy). When presented for a logistic regression analysis, the only remaining variable was abortions in the herd (OR 27; CI 5.958 – 123.795).

Introduction

Bovine brucellosis is a highly contagious disease caused by *Brucella abortus* bacteria, a facultative intracellular pathogen that causes persistent infection in animals (Godfroid et al., 2004). *Brucella abortus* is the usual cause of brucellosis in cattle but *B. melitensis* and infrequently *B. suis* have been implicated. (Anka et al., 2014). It is often characterised by mid to late term abortion and infertility in cows and occasionally orchitis and inflammation of the accessory sex glands in bulls (Godfroid et al., 2004). Abortions, decreased calving percentage, stillbirths, birth of weak calves and decreased milk production often leads to high economic loses for the farmer (Alhaji et al., 2016). Brucellosis affects many animal species especially cattle, sheep, goats, and pigs and also camels, buffaloes, yaks and reindeer (Corbel, 2006).

Brucellosis is an occupational risk for people working with breeding cattle and threatens both food security and food safety. It is a major zoonotic disease worldwide and more than half a million new cases are reported every year (Godfroid et al., 2010). The human disease usually manifests itself as an acute febrile illness which may persist and progress to a chronically incapacitating disease with severe complications (Corbel, 2006). Humans get exposed to brucellosis by consuming unpasteurised milk and milk products, coming into contact with infected material such as uterine contents and inhalation of infected aerosolized particles (Ron et al., 2013). Infected people are subjected to long term antibiotic treatment and take a long time to recover (Corbel, 2006). Brucellosis remains one of the priority diseases because of its presence in many countries and its impact on several animal species (McDermott et al., 2002).

Brucellosis causes losses to livestock owners and the state through direct production losses, culling and costs incurred in disease control and eradication (Mekonnen et al., 2010). The brucellosis situation in the Eastern Cape, South Africa, and other provinces, requires investigation to assist veterinary authorities to implement strategies that can control or eradicate the disease from the provinces. There is active surveillance annually and passive surveillance throughout the year in Eastern Cape. Strain 19 & RB51 are the only *Brucella* vaccines currently approved for use in cattle in South Africa. Statutory use of S19 is limited to the single inoculation of heifers between the ages of four to eight months. Currently, knowledge of risk factors for brucellosis on commercial and non-

commercial farms in Eastern Cape is lacking and needs to be updated The same study was conducted in other provinces and the results will be combined to develop a more comprehensive study with greater power. The findings will assist to modify and enhance the brucellosis eradication scheme where necessary.

Literature Review

Evans recognised the similarity of the agent of Malta fever reported by Bruce to *Bacterium abortus*, the cause of contagious abortion of cattle described by Bang in1897 and the abortus-like bacteria isolated from swine abortions by Traum in 1914 (Banai et al., 2010). In 1886, David Bruce isolated the causal agent, originally called *Micrococcus melitensis* that caused abortion disease of goats, from spleens of infected soldiers on post-mortem. Several patients who were hospitalised had consumed raw milk (Lefevre, 2010). In 1895 Professor Bernard Bang of Denmark isolated the cause of abortion disease of cattle which he named *Bacillus abortus* (Dua, 2012).

Brucella organisms are classified as Alphaproteobacteria, order Rhizobiales and family Brucellaceae (Godfroid et al., 2011). It is a facultative intracellular gram negative coccobacillary organism (Olsen & Tatum, 2010). Traditionally the genus *Brucella* consisted of six species: *B. abortus, B. melitensis, B. suis, B. ovis, B. neotomae* and *B. canis* (McVey et al., 2013) but other species were discovered later; *B. pinnipedalis* (seals), *B. ceti* (cetaceans), *B. microti* (voles) and *B. inopinata* (not known but isolated from breast implant; Banai & Corbel, 2010). Another type of *Brucella* organism was isolated from baboons that had stillbirths and a subcommittee on *Brucella* taxonomy proposed the name *Brucella papionis* sp. nov. (Whatmore et al., 2014). See Table 1 below.

Brucella abortus is the usual cause of bovine brucellosis. In some countries, particularly in southern Europe and western Asia, where cattle are kept in close association with sheep or goats, infection can also be caused by *B. melitensis* (OIE Terrestrial Manual, 2018). No signs of abortions or spread to other animals have been reported for *B. suis* but it may cause a chronic udder infection in cattle (Ewalt et al., 1997).

Table 1 *Brucella* species and their hosts (Whatmore, 2009, Banai & Corbel, 2010)

Species	Biovars	Major Hosts
B. abortus	1-6, 7, 9	Cattle and bovidae
B. melitensis	1-3	Sheep, goats
B. suis	1-5	Pigs, hares, reindeer, rodents
B. canis		Dogs
B. ovis		Sheep
B. neotomae		Rodents
B. pinnipedialis		Seals
B. ceti		Dolphins (cetaceans)
B. microti		Voles (microti avails)
B. inopinata		Unknown (found in breast implant)
B. papionis		Unknown

The highest prevalence of brucellosis has been reported in the Middle East, sub-Saharan Africa, the Mediterranean region, Peru, India, Mexico and China. Several countries in Western and Northern Europe, Australia, Japan, Canada and New Zealand are brucellosis free (OIE, 2018). No accurate figures are available for the prevalence of brucellosis in southern Africa but the introduction of compulsory calfhood vaccination in South Africa resulted in a decline from about 10.5% in 1976 to 1.4% in 1988 (Godfroid et al., 2004).

Table 2 Common diseases caused by *Brucella* spp *and affected livestock.*

***Brucella* species**	**Disease**	**Livestock Species**
B. abortus	Brucellosis (contagious abortion)	Cattle and bovinae
B. ovis	Epididymitis/orchitis	Sheep
B. melitensis	Abortion and orchitis	Sheep and goats
B. suis	Abortion, stillbirth, sterility in sows and orchitis	Pigs

Studies on brucellosis prevalence are based mainly on serology. Most surveys are on cattle brucellosis, occasionally for sheep and goats and rarely for pigs (Macdermort & Arimi, 2002).Transmission of brucellosis is usually through direct or aerosolised mucosal contact with bacteria in fluids or tissues from aborted or birth material (Olsen & Tatum, 2010). Susceptible animals may ingest contaminated grass, feed and or water or lick contaminated genitals of other animals (OIE Terrestrial Manual, 2018).

The identification of herd level risk factors could allow for effective disease management, even in cases where the epidemiology of brucellosis is not clearly understood. Known risk factors for brucellosis include larger herd sizes, the purchase of breeding stock, and pasture rental (Cowie et al., 2014). An Italian study showed that contact with sheep and goats was the major risk factor as well as large herd size and *B. melitensis* was isolated from most of the positive herds in contact with sheep and goats (Dalla Pozza et al., 1997). Ninety one cases of *B. melitensis* were reported to the OIE in South Africa between 1996 and 2000 (McDermot & Arimi, 2002).

A study in Zimbabwe showed that all six smallholder dairy herds had *Brucella* seropositive dairy herds. Some of the risk factors identified were herd size, stocking density, geographical area and cattle breed. Imposing movement controls, and avoiding mixing different breeds could help decrease seropositivity (Matope et al., 2010). A Brazilian study described how the large size of the female population in the herd markedly increased the risk of disease compared with smaller herds, other factors being extensive cattle production and purchase of replacement stock from traders or directly from other farms. The authors recommended high vaccination coverage of heifers (De Oliveira et al., 2016). In Northern Ireland, direct contact between cattle at pasture was the most likely means of between-herd transmission for most (71%) outbreaks, with an attack rate of 28.1% in herds immediately neighbouring the primary outbreak herds and 11.3% in the next concentric ring of farms. Control of the outbreak was achieved through a quick response by the veterinary officials, outbreak investigations, continuous testing of high risk herds and parallel testing of herds (Abernethy et al., 2011). Other factors associated with brucellosis occurrence are extensive movement of cattle, mixing while grazing and at water sources (Kadohira et al., 1997). Studies need to be done in South Africa to manage the endemic brucellosis challenge.

Brucellosis can infect humans and cause undulant fever or Malta fever (OIE, 2018). Clinical signs in humans include intermittent fever, anorexia, sweats, joint pain, headache, pneumonia and endocarditis (Sauret & Vilissova, 2002). In some cases the liver, spleen and other organs may be infected (OIE, 2018)**.** Humans are exposed when they consume unpasteurised milk and milk products or when in contact with infectious material such as uterine contents, aborted foetuses and infected carcasses (Alcina et al., 2010). Those working with infected animals such as veterinarians, farm and abattoir workers may get infection orally, via the respiratory route or through the eyes (Lopes et al., 2010) It is rare for the disease to be passed from human to human (Godfroid et al., 2011).

The facultative intracellular parasitic behaviour of *Brucella* species has evolved by evolutionary selection to evade the host immune system. Bacteria invade the digestive tract by epithelial transmigration of bacteria, preferentially through M cells. *Brucella* may also be transported by intra-epithelial phagocytes from the intestinal lumen to the lamina propria (Xavier et al., 2010). *Brucellae* target trophoblasts, foetal lungs, macrophages and reproductive organs (Poester et al., 2013). High concentrations of steroid hormones and erythritol enhance the growth of *Brucellae* inside trophoblasts (Xavier et al., 2010). *In-utero* infection causes placentitis, leading to a disturbance of gaseous exchange between dam and foetus, resulting in the death of the foetus and abortion (Schlafer & Miller, 2007). Large quantities of *Brucella* organisms are excreted in the placenta, foetal fluids and vaginal discharges (OIE Terrestrial Manual, 2018). Sometimes placental lesions are mild, causing weak newborn calves and resulting in a high neonatal death rate (Schlafer & Miller, 2007). Persistence of the bacteria in macrophages results in chronic infections that are characteristic of brucellosis in different host species (Roop et al., 2009).

Extreme caution must be taken when handling *Brucella*-suspect specimens because of its zoonotic nature. In abortion cases, a whole foetus may be submitted if feasible or foetal stomach contents, foetal lesions, uterine discharges, cotyledons, colostrum or paired serum samples (Markey et al., 2013). Diagnostic methods used for brucellosis include direct detection, involving bacteriological culture or by polymerase chain reaction (PCR)-based methods and indirect methods, which are tests done on milk or blood or serum and in some instances skin allergic tests (Godfroid et al., 2010). Isolation of the organism provides a definitive diagnosis (OIE Terrestrial manual, 2018).

A presumptive diagnosis can be made by assessing serological or cell-mediated responses to *Brucella* antigens (OIE Terrestrial Manual, 2018). Culture and biotyping can be used to distinguish vaccine reactions and *B. abortus* field infection. Most standard brucellosis serologic tests such as agglutination, complement fixation, fluorescence polarisation assay and enzyme-linked immunosorbent assay (ELIZA) use the polysaccharide O-chain from *B. abortus* as antigen and were initially developed for identification of *B. abortus* organisms in cattle (McVey et al., 2013). Serological reactions cannot distinguish field strain infections from S19 vaccine reactions (Godfroid et al., 2010).

Table 3 **List of** direct and indirect diagnostic test for bovine brucellosis

Direct Diagnostic tests		**Indirect Diagnostic tests**
		Antibody Detection
Smears (foetal organs, cotyledons, uterine discharges)	Culture (foetus, placenta, uterine discharge, colostrum, milk, semen, lymph nodes)	Rose Bengal Test (serum)
		Serum agglutination test (serum)
		Complement fixation test (serum)
		Flouresence polarisation (serum or blood)
		Milk ring test (milk)
		Intradermal skin test (unvaccinated calves; latent – cellular)

Although isolation of *B. abortus* remains the ‘gold standard’ for diagnosis, the true sensitivity of culture for individual animals remains unknown (O’Grady et al., 2014). Screening tests used locally or nationally include the rose bengal test (RBT), buffered plate agglutination test (BPAT), ELISA and flouresence polarisation assay (FPA). Those that test positive are re-tested using a suitable confirmatory test (OIE Terrestrial Manual, 2018). In South Africa, the Department of Agriculture Forestry and Fishery (DAFF) recommends the complement fixation test (CFT) because of its high sensitivity and specificity. The CFT, however, cannot distinguish S19 reactors and field strain when recent and repetitive vaccinations are used in old heifers and adult cattle. Because the CFT is difficult to standardize, it is progressively being replaced by ELISAs. This test is a ‘prescribed test for trade’ by the OIE (Godfroid et al., 2010). In South Africa, according to

the Bovine brucellosis manual published by Department of Agriculture in 2016, the disease is controlled in terms of the Animal Diseases Act, 1984 (Act 35 of 1984). Tests used are direct diagnostic methods which include smears and culture and indirect methods such as RBT , SAT, CFT, ELISA, MRT and brucellin test.

Brucellosis control and eradication programmes are designed to limit transmission of the disease among animals and also to humans. The programmes prevent economic losses associated with infertility, foetal loss and reduced milk production (Olsen & Tatum, 2010). For the programme to be effective, situation analyses and needs assessments must be conducted. This can be achieved through epidemiological surveys and assessing the significant risk factors, knowledge, attitudes and practices (KAPs) of the farmers (Smits, 2013). Control in South Africa is based on providing the animals with effective immunity and removing infected animals from the herd timeously to prevent spread of infection to clean stock.

Treatment of brucellosis cases is not allowed in many countries because of the potential to result in a carrier status of treated animals and to limit antibiotic resistance in food animals (Lefevre, 2010). An effective control strategy will ensure that animals acquire adequate immunity (vaccinations), infected animals are removed from susceptible herds timeously (test and slaughter) to limit spread of infection, a surveillance programme is put in place supported by an adequate veterinary infrastructure and proper animal movement control is implemented (Lefevre, 2010; McVey, 2013). Replacement stock must originate from certified *Brucella* negative herds. On arrival at a farm, they should be isolated for about 30 days and retested before introduction to the herd (Dua, 2012).

Vaccination is a very important aspect in brucellosis control programmes of livestock, especially as there is no successful treatment available (Dua, 2012). Three vaccine strains of *B. abortus* have been used in animals: strain 19, a smooth strain, used as a live attenuated vaccine, strain 45/20, as a rough killed vaccine and, more recently, strain RB51, a rough live attenuated vaccine. In South Africa only strain 19 and RB51 are currently allowed to be used in cattle (Godfroid et al., 2010). Control of human brucellosis relies on control in the animal reservoir since there is no vaccine for humans (Godfroid et al., 2011).

S19 vaccine induces good immunity (OIE Manual, 2018) and statutory use of S19 vaccine in cattle is currently limited to the single vaccination of heifers from four to eight months of age. Booster vaccinations with RB51 vaccine will induce an improved and prolonged immunity. Strain 19 & RB51 are the only Brucella vaccines currently approved for use in cattle in South Africa. In Eastern Cape province, calfhood vaccinations are done on heifers at four to eight months of age. There is annual active surveillance and passive surveillance throughout the year.

The RB51 strain vaccine is a rifampicin-resistant mutant of *B. abortus* strain 2308 and is essentially devoid of the O-polysaccharides (Lefevre, 2010). It is a rough attenuated strain that does not induce antibodies specific to the O-chain in quantities measurable by classical serological tests, even after injection of adult females or repeated injections (Lefevre, 2010; Dua, 2012). It is less likely to induce abortion in pregnant cows than S19. A reduced dose of RB51 protects adult cattle against infection and abortion caused by the exposure to a virulent strain (Herrera-López et al., 2010).

South Africa has long been known to have brucellosis. From 1996 to 2004, between 291 and 457 outbreaks of bovine brucellosis were reported yearly to the OIE. Brucellosis has a high prevalence in southern Africa, especially in farms practising intensive agriculture, and causes huge losses to farmers (Hesterberg et al., 2008). A survey of about 90% of the dairy and beef herds in the Eastern Cape Province and Karoo between 1985 and 1989, revealed a prevalence of less than 0.3% (Godfroid et al., 2004).

Materials and Method

This study is one of several that are being conducted concomitantly using the same methodology and questionnaire. Others are being undertaken in KwaZulu-Natal, (Nogwebela pers comm) and in Gauteng Province (Govindasamy, pers comm).

This study was approved by the Department of Rural Development and Agrarian Reform in the Eastern Cape Province of South Africa. A consent form was read and signed by the participants before administering the questionnaire (Appendix 1). No live animals were used in this study. Ethics approval is attached (Appendix 2).

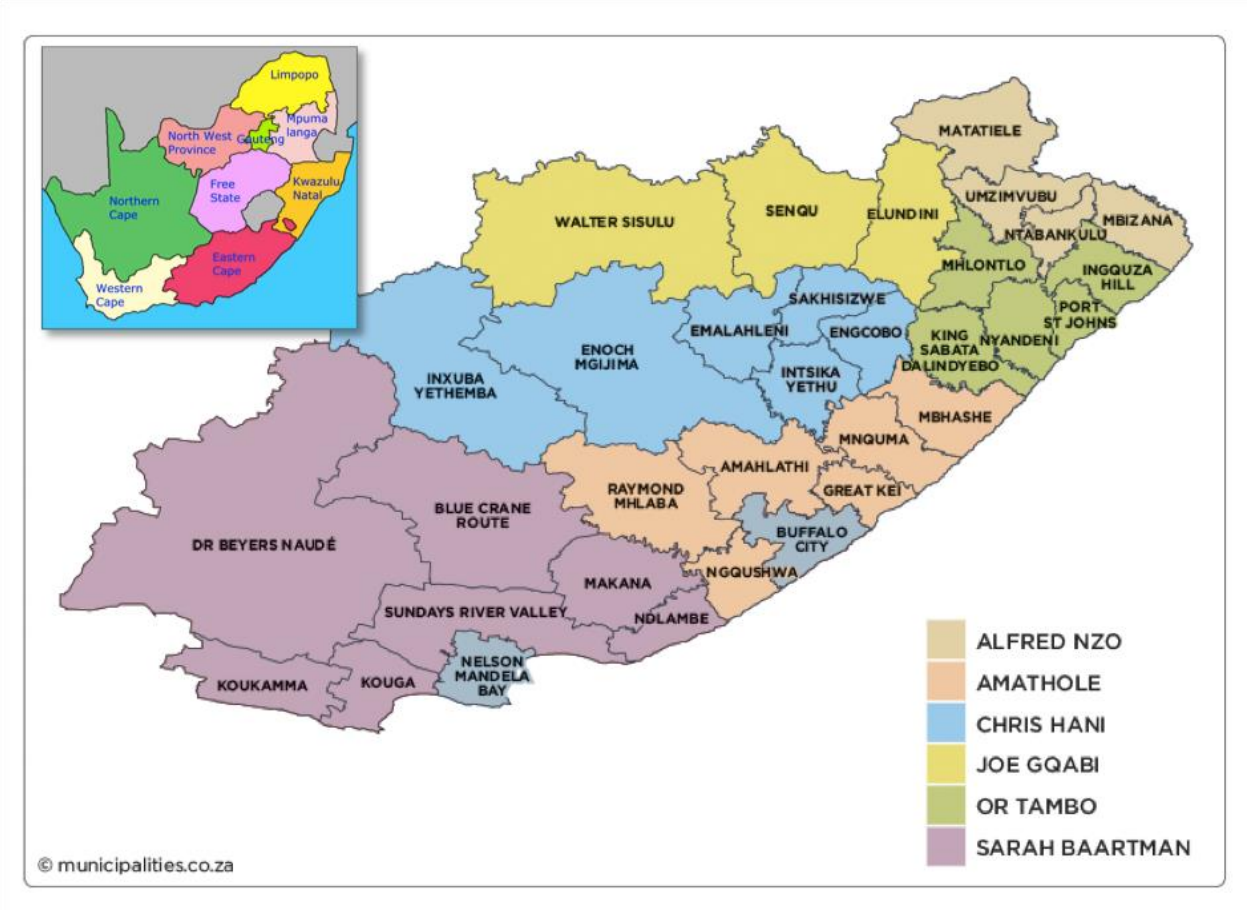

Figure 1 District municipalities of Eastern Cape, South Africa, National Government

The case control study was done in the Eastern Cape province of South Africa. The Eastern Cape is located on the east coast of South Africa between the Western Cape and KwaZulu-Natal provinces. Inland, it borders the Northern Cape and Free State provinces, as well as Lesotho. The province has six district municipalities which are sub-divided into 31 local municipalities (Figure 2). Case herds were reported in five out of the six districts and therefore the studies were done in these five districts. The districts are Alfred Nzo, Amathole, Chris Hani, Joe Gqabi and Sarah Baartman. OR Tambo district had a few reactor cattle but they were found to be S19 reactions.

The state veterinary service directorate in Eastern Cape carry out annual surveillance of brucellosis on heifers, cows and bulls over 18 months of age. Government-employed animal health technicians bleed animals at various farms. Vaccination of heifers from four to eight months of age with S19 in the various state veterinary areas. Suspect *Brucella* cases (abortions, retained placentas etc) are also tested for Brucellosis and other related diseases such as Rift Valley fever throughout the year. Information was collected from the provincial veterinary head office and three veterinary laboratories in the province on the number of cases that were reported for bovine brucellosis between September 2013 and January 2017. A case control study design was used. Case herds were defined as those with at least one culture-positive animal or more than two CFT-positive reactors, in the absence of adult Strain 19 vaccination, between September 2013 and January 2017. Control herds were defined as those that tested negative within six month of case herds and had no history of brucellosis. For every case, two controls were randomly selected from the same state veterinary area. Some cases and control herds were latter dropped because of delays or refusal to have an interview.

In Eastern Cape Province, all districts and state veterinary areas take part in the annual surveillance programme for brucellosis in both commercial and non-commercial farms. The sampling frame in this study included all herds tested in the province during the study period from September 2013 to January 2017. Most of the herds were tested annually although there were little variations in the different districts. All bovine *Brucella* positive herds were included in the survey and control herds were sampled using the positive herds' spatial and temporal distribution. The data for cases were obtained from provincial disease reports, state veterinarians reports and personal communication and provincial laboratories results data. Suspect cases were also sampled and sent to the provincial laboratory for bacteriological and serological testing. Most of the abortion samples sent

to the laboratory were tested for brucellosis. Routine tests conducted at the laboratory included bacterial culture, Rose Bengal test (RBT), serum agglutination test (SAT), complement fixation test (CFT) and milk ring test. CFT was used in most cases as a confirmatory test. To decide if an animal was positive, several factors were put into consideration like the animal history, titres, different tests results, S19 vaccination reactions and herd status.

A total of 77 farms were recruited for the study, comprising 30 cases and 47 controls.The intention was to have 2 controls for each case farm but budgetary constraints and resistance by some resulted in only 47 control farms being recruited. Five potential case farms could not be recruited because the owners did not consent to be interviewed and others had ceased operation at the time of the study. Interviews for this study were conducted on the farm by trained animal health technicians and state veterinarians using a pre-tested questionnaire (see annexure). Assessed risk factors included herd characteristics, cattle movements, potential brucellosis contacts, presence of wildlife and management/employee knowledge and the health aspect of the farmer, his or her family and employees. Despite the few numbers of cases in the province, there was a need to go ahead with the study to complement the study that was done concurrently in KwaZulu-Natal and Gauteng so that the findings could be combined for a comprehensive study for publication.

Data analysis

Data were transferred to an Access 2013 database and analysed in Excel 2013 and SPSS (IBM, Version 25). Unadjusted odds ratios were calculated using univariable logistic regression analysis and adjusted estimates from a multivariable logistic regression.

Upon completion of the univariable analysis, all variables with a probability value $P < 0.25$ were allowed to go forward to the logistic regression using the ‘Enter’ method in SPSS. For a variable to enter the model the probability was set at $P < 0.05$ and for a variable to leave the model the probability was set at $P > 0.1$. Linearity of continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. Extreme values of herd size (> 1500, n=3) were omitted from the analysis although neither this, nor log-transforming the variable significantly affected the final

model results. Studentized residuals were used to test for outliers and those with residual values greater than 2.5 standard deviations were inspected in detail. The overall goodness of fit of the final model was assessed using the Hosmer-Lemeshow test. The final step in the analysis was the calculation of odds ratios with corresponding 95% confidence intervals.

Results

Descriptive

Thirty *Brucella* case-herds and 47 controls were recruited within the study period (September 2013 to January 2017) with 81.8% in 2015 or 2016. The number of cases per municipality varied from zero to 14 (Figure 2).

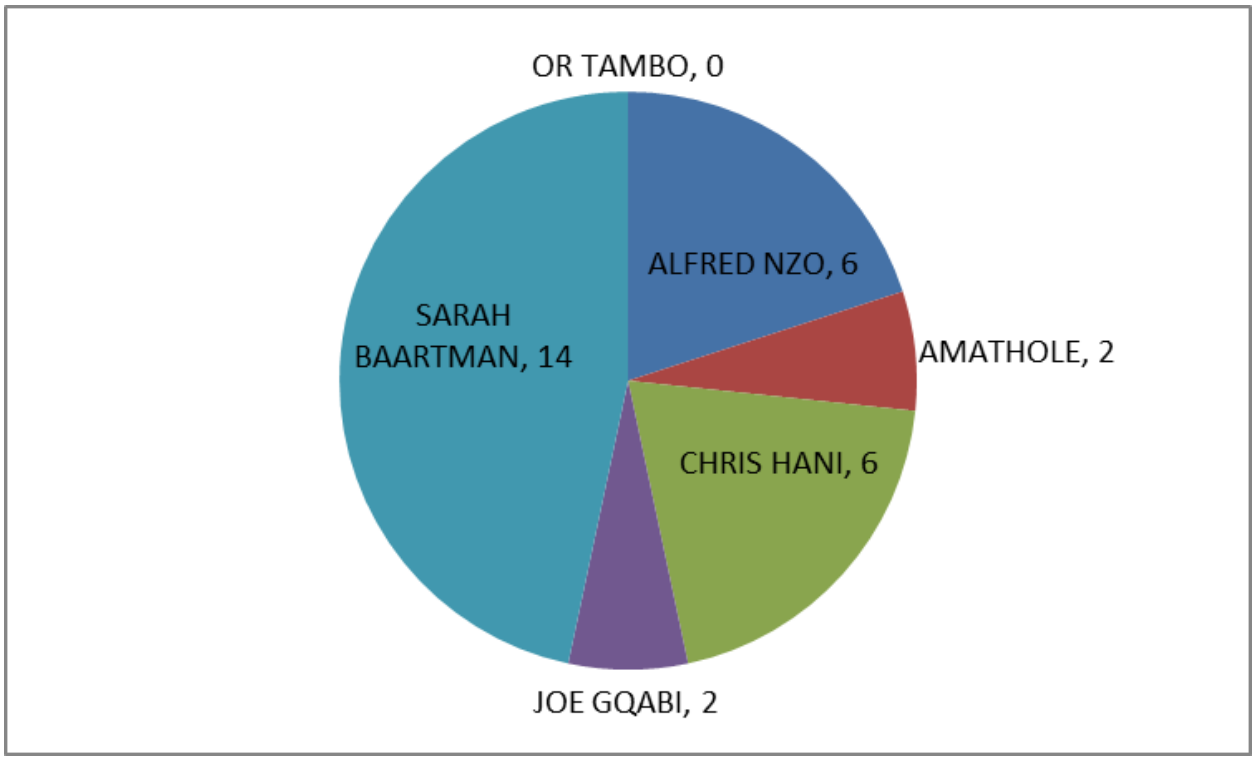

Figure 2 Pie chart showing the distribution of cases in the Eastern Cape province

Sarah Baartman, Chris Hani and Joe Gqabi are predominantly commercial farms whereas OR Tambo, Alfred Nzo and Amathole are predominantly communal farms.

The median herd size at interview was 105 cattle (range 2-4000) with case herds being larger than control herds: for the former, median = 138.5, Tukey's hinges = 34 and 543 while the median size in control herds was 59; Tukey's hinges = 24.5 and 385 (Figure 4). The median number of cows was also higher in cases compared to the controls (93.5 v

23). Eleven herds (14.3%), comprising six controls and five cases, were larger than 1000 cattle and accounted for 64.7% of the cattle in the study population.

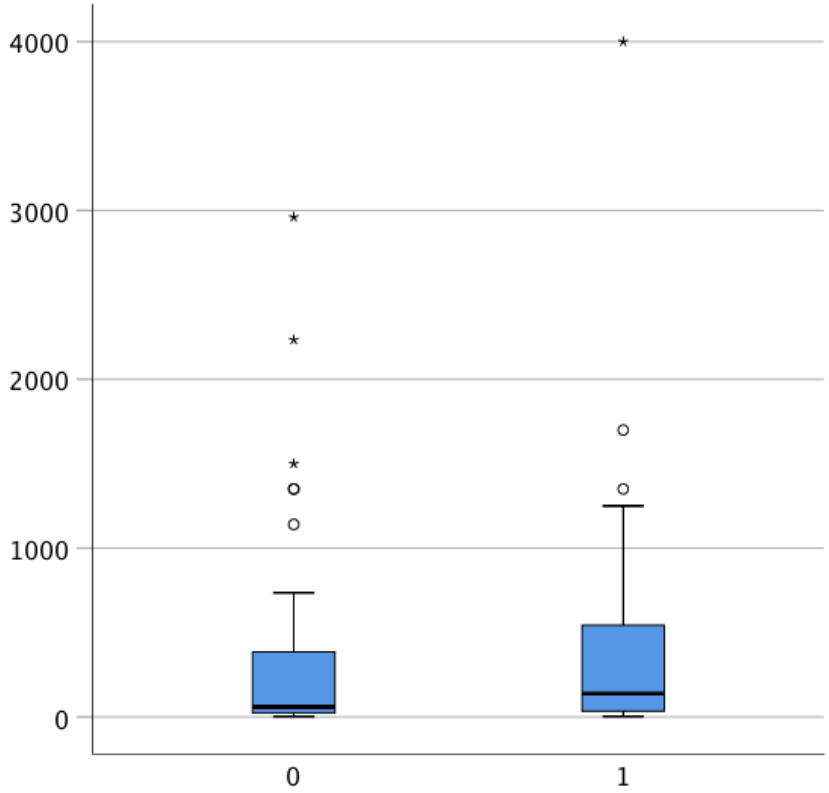

Figure 3 Box plot of herd size for control (=0) and case (=1) herds

Eighty eight percent of the interviewees (n=154) were the owners of their herds, 8.6% were employees while 3% were managers.

In respect of management issues, 18 of 30 (60%) respondents on case farms reported that their *Brucella*-positive cattle had not been branded. Only 25% (6/24) of all herds that received new cattle had them tested for brucellosis. A third of the case farm owners and 19% of control herd owner received brucellosis training while equivalent data for farm workers was 26.7% and 6.4% respectively. Ninety five percent of the respondents wanted further training on brucellosis. 26.7% of case herds reported *Brucella*-suspicious symptoms in their personnel whereas only 12.8% of control herds reported symptoms. One case herd farm owner tested positive to brucellosis but this finding was not part of the questionnaire.

Univariate analysis

The univariable analysis revealed seven variables with p values ≤ 0.05. There was an increasing risk with increase herd size, although this was not statistically significant. Conversely, the proportion of female cattle in the herd was protective, although this was only statistically significant in the stratum of highest proportion (>0.64; Table 4). Herd type (commercial/communal and dairy/non dairy) was statistically significant and associated with an increased risk as was the presence of abortions, the use of AI and neighbours that had experienced a brucellosis outbreak. In respect of training, for either owners or workers were also associated with an increased risk but only the latter was significant.

Table 4 Results of the univariable logistic regression analysis for a farm to be positive for brucellosis.

Variable	Stratum (Number herds)	% Cases	Odds Ratio	95% C.I.	p-value
Herd Size	1-28 (n=25)	36.0	1	-	-
	29-152 (n=23)	30.4	1.1	0.255-4.262	0.955
	153-4000 (n=29)	48.3	2.4	0.763-7.397	0.136
Proportion of Cows/Heifers	<0.45 (n=24)	75.0	1	-	-
	0.46 - 0.64 (n=27)	51.9	0.4	0.109-1.184	0.092
	>0.64 (n=26)	38.5	0.2	0.062-0.703	0.011
Commercial v Communal			3.4	1.262-9.188	0.016
Dairy v Non-dairy			2.9	1.105-7.699	0.031
Sheep or goats present (Y/N)			0.4	0.153-1.114	0.081
Inward movement of cattle (Y/N)			1.5	0.568-4.033	0.407
Abortions in herd			21.5	6.007-76.951	< 0.001
Neighbouring herd *Brucella* positive			4.7	1.603-11.878	0.004
Wild ruminants on neighbouring farm			2.5	0.799-7.506	0.117
Workers have own cattle (Y/N)			1.6	0.307-8.661	0.567
Use of a bull (with/without AI)			1.7	0.302-9.198	0.558
Use of AI (with/without bull)			6.0	1.956-18.276	0.002
Brucellosis symptoms in people (Y/N)			0.9	0.410-1.925	0.764
Cattle fenced in (Y/N)			1.4	0.490-3.862	0.610
Owners received training in brucellosis control (Y/N)			3.0	0.950-9.477	0.061
Workers received training in brucellosis control (Y/N)			4.7	1.136-19.677	0.033

Multivariable logistic regression

The logistic regression model was statistically significant, χ^2 (11) = 58.2, $p < 0.0005$, explained 76.2% (Nagelkerke R^2) of the variance and correctly classified 88.7% of cases. The sensitivity was 85.2% and specificity was 90.9%. Of the eleven predictor variables only the presence of abortions was statistically significant (OR = 27.2 95% CI = 6.0-123.795; $p < 0.001$).

Discussion

This study was the first bovine brucellosis case control study to be undertaken in the Eastern Cape Province. Accordingly, it provided novel information on herd level risk factors for bovine brucellosis occurrence s well as a procedure for investigating such incidents.

The presence of abortions was strongly associated with an increased disease risk and the odds of an outbreak increased when adjusted for other variables. Such an association is consistent with studies elsewhere: Kumar et al. (2005) found a threefold increase in prevalence given a history of abortions (33.87% v 11.63%), while studies in Peninsular Malaysia (Anka et al., 2014) and Uganda (Makita et al., 2011) reached similar conclusions. Why is abortion such a big risk? Abortions – and infected parturitions – result in a massive release of organisms, where a single episode may produce 10^8 infective doses and one micro litre of the latter may contain an infective dose (Alton, 1983). Thus, the potential for spread to contact animals is extremely high, especially if cattle are overcrowded, as in most dairy herds in Eastern Cape. Furthermore, *Brucella* organisms can survive in an aborted foetus in the shade and also in liquid manure stored in tanks for up to eight months, three to four months in faeces, two to three months in wet soil and one to two months in dry soil (Godfroid et al., 2004). Thus, although bacteria will dessicate quickly in the hot African sun, they may persist in damp, moist conditions for prolonged periods unless proper disinfection methods are used.

Abortions are not currently notifiable in South Africa; making every abortion a notifiable event will go a long way in alerting veterinary authorities and limit spread of the disease within herds and to neighbouring farms. Such a system will also provide invaluable surveillance for other diseases causing bovine abortion such as Q fever or Rift Valley fever (Bronner et al., 2014) as well as less common zoonotic diseases. A specific set of samples from each abortion (e.g. blood or serum, foetus or foetal lungs and vaginal swabs) should be submitted to a diagnostic laboratory for every abortion case. Care should be taken on handling and disposal of aborted material, as it may expose humans and animals to infection.

Brucellosis in a neighbouring herd was associated with a fourfold increase in risk although this was not statistically significant in the final model. Neighbouring farms were considered as those sharing at least one external boundary. The lack of significance may be attributed to the few cases and controls used in this study, i.e. lack of power. In Northen Ireland, direct contact between cattle at pasture was identified as the most likely means of between-herd transmission for most (71%) outbreaks, with an attack rate of 28.1% in herds immediately neighbouring the primary outbreak herds and 11.3% in the next concentric ring of farms (Abernethy et al., 2011). In the Eastern Cape, communal grazing may have resulted in significant between-herd contact; 31% of interviewees did not have a perimeter fence, but even in commercial herds - which should operate as discrete units, there was still a high chance of contact between animals of neighbours and an abortion would put susceptible animals at risk. An intact perimeter fence may reduce but not eliminate spread. Infected cow's aborted material and afterbirth can spread the disease easily to neighbouring farms when they mix. Once a herd has been diagnosed with brucellosis, awareness must be done to surrounding neighbours and the whole community at large. Slaughter and quarantine of animals should be enforced.

Use of artificial insemination with or without a bull was found to be significant in the univariate but was not significant in the multivariate analysis. In an Ethiopian study, artificial insemination was shown to be a significant risk factor for bovine brucellosis (Jergefa et al., 2009). Farmers practising artificial insemination need awareness of the risk of acquiring brucellosis from infected semen. Source of semen should be checked for brucellosis-free status before purchase. Semen, seminal fluid and urine from infected bulls may shed *Brucellae* and therefore in infected herds they should not be used, particularly if artificial insemination using their semen is contemplated (Godfroid et al., 2004).

Commercial farming in Eastern Cape was found to be a higher risk compared to communal farming, although the finding was not statistically significant. Previous studies ave found that practising intensive farming in commercial farms tends to promote the transmission and persistence of *Brucella* spp. infection especially following abortions (Matope et al., 2010). The communal farmers tend to keep their herds closed for years without introducing new stock. They also buy locally and this limits the risk of getting *Brucella* from positive herds. The state veterinary services also help communal farmers with S19 vaccine for heifers coupled with annual brucellosis surveillance and this is done

by qualified animal health technicians. Eastern Cape has one of the largest numbers of animal health technicians and state veterinarians providing services to communal farmers. An example is Alfred Nzo District municipality area which is predominantly rural and, at the time of the survey, had four state veterinarians and 49 animal health technicians. In commercial areas however, a few animal health technicians are allocated to them and they depend mainly on private veterinarians.

The odds of *Brucella* positivity was found to be higher in dairy cattle than in non-dairy herds but it was not statistically significant. Dairy cattle was defined as a herd with only dairy cattle in the herd while non-dairy herds did not have dairy cattle in the herds. The reason for the higher prevalence in dairy herds can be attributed to larger herd size, high stocking density, high percentage of females and improved surveillance by use of milk ring tests. Because of the risk of people getting brucellosis from consuming infected milk, surveillance of dairy herds must be made mandatory to limit the spread to people and animals.

Herd size has always been a significant risk factor in many studies of bovine brucellosis (Muma et al., 2006; Matope et al., 2010; Makita et al., 2011). In this study it was observed that there was an increase in the risk with increase in herd size but it was not statistically significant, again likely due to the lack of power. In a serological survey done in Ivory Coast, the odds of brucellosis seropositivity for herds with more than 100 cattle was 3.3 (95% CI: 1.2, 8.9) times higher compared to those with less than 50 cattle (Sanogo et al., 2012). Larger herds usually have higher stocking densities and a higher probability of increased exposure to infected animals or contaminated materials.

Training of owners and workers can go a long way in reducing the spread of the disease before and after outbreaks. A third of the farm owners and 26.7% of employees received training for brucellosis management in case herds whereas it was 19% and 6.4% respectively for control herds. The higher percentage among case herds was mainly because of after outbreak awareness. Ninety five% of the farmers expressed their willingness to get further training on brucellosis prevention and control. The state and private veterinary services should pull their resources together to assist farmers to get an understanding of this zoonotic and economically significant disease and how to prevent and control it. Training and refresher courses for veterinary staff may be needed to

mobilise and implement a campaign on farmer education on bovine brucellosis and other infectious diseases.

It is noteworthy and a matter of concern that 60% of positive herds did not C-brand their positive cattle. Non-branding can promote spread of disease as cattle may be resold to unsuspecting farmers or moved to naïve herds. Generally, infected cattle are sold at a cheaper price so a farmer may decide to sell them without disclosing their status. In South Africa, bovine brucellosis is a controlled disease in accordance with the Animal Diseases Act (Act 35 of 1984) and the Animal Disease as well as the Bovine Brucellosis Scheme Regulations. There is a need to enforce the regulations by the veterinary authorities to ensure that farmers do not do as they please. Only 25% tested their new stock after arrival. Failure to test introduced animals can put the herd at risk especially when infected heifers and cows start to give birth or abort.

It was interesting to note that of the five government sponsored herds, none of them were case herds. This can be attributed to strict state procurement regulations and testing before arrival on the farm of recipient. It may also be a result of few government sponsored herds leading to few chances of detection since abortions or suspected animals are not reported or samples send in for testing. If the state ensures that all animal purchased meet the breeding and soundness evaluation, and serosurveillance for brucellosis, then the risk of disease transmission can be minimised. In *Brucella*-free countries or regions, surveillance should include testing before and after movement and brought-in animals must be quarantined. To limit cross border introduction of the disease regular testing should be done at areas adjacent to porous borders (Ndengu et al., 2017), on animals imported for breeding purposes and also semen, embryos and ova (Robinson, 2003).

This study provided information that can be used to assist government strategies but there are several shortcomings that need to be highlighted. Based on multivariable modelling, only presence of abortions is considered a risk factor. The number of cases and controls were few and this likely reduced the power of the study. This was mainly because the cases reported during the period under study were very few and consent from the farmer was required before proceeding. The study area was huge and this created logistical and financial challenges. Personal interviews were costly to organise, involving training, payment and travelling expenses of interviewers. Because farmers

were asked about events that happened in previous years, there is likely to be a recall bias. Since all cases were included in the study with the exception of those who would not consent and those who had closed operation after the outbreak, there was selection bias. Misclassification, though rare, may occur as a result of S19 reactors that could not be picked on rebleeding.

Nonetheless, the study findings will be useful to assist the state authorities in targeting resources more effectively and determining control strategies specific to Eastern Cape as well as outside the province.
More studies need to be done to understand how the disease continues to occur despite efforts to reduce and eradicate the disease.

Conclusion

Bovine abortions should be taken seriously as they pose a high risk to the cattle and human population in the Eastern Cape Province. All bovine abortions, therefore, should be made notifiable to the state veterinarian, and a *Brucella* test should be done on all abortions. Since 95% of farmers need further information on brucellosis, resources should be made available to educate farmers and farm workers on bovine brucellosis prevention, control and the zoonotic implications. The veterinary regulations pertaining to brucellosis should be enforced by government officials to minimise illegal movement of infected animals and eventual spread. Enhanced passive and active surveillance is crucial to ensure that sources of infection are traced, isolated and removed. The number of cases in the current study can be improved by recruiting additional cases and control in future to increase the power of the study.

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Annexure 1: Copy of Questionnaire

Case Control Study of Risk Factors for Brucellosis

INFORMED CONSENT

Dear Participant

This study is undertaken on behalf of postgraduate students from the Department of Veterinary Tropical Diseases, University of Pretoria. You are invited to participate in this survey:

Case Control Study of Risk Factors for Brucellosis

This letter gives information to help you to decide if you want to take part in this study. Before you agree you should fully understand what is involved. If you do not understand the information or have any other questions, do not hesitate to ask us. You should not agree to take part unless you are completely happy about what we expect of you.

The purpose of the survey is to determine the risk of herds like yours getting or maintaining Brucellosis. Brucellosis is a bacterial disease that can cause abortions in affected herds, which reduces milk and beef production and thus causes a loss of income. It is also dangerous to people, causing flu-like symptoms that can occur for life.

Your participation will help us to better understand what are the main drivers of the disease and thereby identify ways of eliminating it.

I am going to ask you some questions that are listed in this questionnaire. The questionnaire will be kept in a safe place to ensure confidentiality. Your identity and the name of your farm will not be used in any analysis or reports. The data will be used for this study and for animal health studies but your name and that of your farm will be kept strictly confidential.

Note: The implication of completing the questionnaire is that informed consent has been obtained from you. Thus any information derived from your form (which will be totally anonymous) may be used for e.g. publication, by the researchers.
We sincerely appreciate your help.

Do you agree to go ahead with the questionnaire? Yes ☐ No ☐

Farm Name _____

Farm owner or Representative Name _____

Signature _____

FOR OFFICE USE ONLY:

(To be filled out & registered on Case Control Database by Investigator before distribution to Field Technician):

(O.2) Farm Study Status (please mark with X):

Case Farm (2 or more serologically positive cattle in herd on confirmatory CFT where vaccination is unlikely to be the cause or culture positive sample)	
Control Farm (A herd where all cattle tested negative within 6 months of a case herd (see above definition) within the same State Vet Area & in the absence of any clinical signs of brucellosis within the herd during those 6 months)	

(O.1) Unique Record #

(O.3) Reference Date
(Date of herd test)

DD/MM/YYYY

(O.4) Laboratory #

YYYYD_____

(O.5) Were cattle vaccinated within the 2 year period before the Reference Date (O.3) Y/N?

(O.6) FARM DETAILS (From CAS/laboratory sample submission form)

O.6.1	FARM NAME + FARM ID			
O.6.2	STREET			
O.6.3	SUBURB			
O.6.4	DISTRICT			
O.6.5	STATE VET AREA			
O.6.6	PROVINCE			
O.6.7	POSTAL CODE			
O.6.8	GPS EAST			
O.6.9	GPS SOUTH			

(O.7) CONTACT PERSON DETAILS (From CAS/laboratory sample submission form)

O.7.1	FIRST NAME	
O.7.2	SURNAME	
O.7.3	CELL NUMBER	
O.7.4	LANDLINE	
O.7.5	EMAIL ADDRESS	

(O.8) INTERVIEWER DETAILS (Please mark with an X who will be conducting the interview. Please fill in the cell number of the person selected)

O.8.1	AHT NAME		
O.8.2	STATE VET NAME		
O.8.3	CELL NUMBER		

(O.9) RECRUITED? (Y/N)

(O.9) INTERVIEW DATE DD/MM/YYYY

TO BE COMPLETED BY AHT/STATE VET CONDUCTING THE FIELD INVESTIGATION

(O.10.1) Is the information captured above (O.2 – O.7) complete AND correct? Y/N

(O.10.2) If No, please complete AND correct

(A) INTERVIEWEE DETAILS

(A.1) PERSON INTERVIEWED (Must be in control/manager of cattle and present during period of outbreak for a case farm)

(A.1.1)FIRST NAME	(A.1.2) SURNAME	(A.1.3) CELL NUMBER	(A.1.4) EMAIL

(A.2) STATUS (Please mark with an X)

A.2.1	OWNER	
A.2.2	MANAGER	
A.2.3	EMPLOYEE	
A.2.4	FAMILY MEMBER	
A.2.5	OTHER, Please specify beneath:	

(B) HERD DETAILS

(B.1) HOLDINGS (Please complete the table below: each row is a holding on which the owner's cattle are kept)

(B.1.1) HOLDING CURRENTLY ON

No.	Farm/Plot Name	Total No. of Cattle	Was Brucellosis diagnosed on this farm within the last 5 years? (Please mark Y=Yes, N=No OR U=Unknown)	Is this a government sponsored farm/project? (Please mark Y=Yes, N=No OR U=Unknown)
1				

(B.1.2.1) IS THIS THE MAIN HOLDING? (Y=Yes, N=No, U=Unknown) ☐

(B.1.2.2) IF NO, PLEASE STATE THE FARM NAME, FARM NUMBER AND PLOT NUMBER OF MAIN HOLDING: FARM NAME:_____FARM NO._____PLOT NO._____

(B.1.3) OTHER HOLDINGS WHERE YOU KEEP YOUR CATTLE

No.	Farm/Plot Name	Total No. of Cattle	Was Brucellosis diagnosed on this farm within the last 5 years? (Please mark with an X. Y=Yes, N=No OR U=Unknown)	Is this a government sponsored farm/project? (Please mark Y=Yes, N=No OR U=Unknown)	Km from B.1.1
2					
3					
4					
5					
6					

(B.1.4) Please mark the above farms/plots on the map provided (Indicate the approximate spatial position using the numbers of the farm from B.1.1 and B.1.2. You can use arrows and mark each arrow with the correct farm/plot number. If it is OUTSIDE the province, please mark the holding # (B.1) OUTSIDE the map border

Eastern Cape province Map. (Please note that Umzimkhulu, on far right, is no longer part of Eastern Cape province.)

(B.2) TYPE OF HERD

B.2	TYPE OF HERD (Multiple herds may be selected. Please mark the appropriate herds with an X)		ON WHICH FARMS/PLOTS MENTIONED ABOVE (B.1.1 & B.1.2) ARE THE SELECTED HERDS FARMED?					
			1	2	3	4	5	6
B.2.1	STUD HERD							
B.2.1.1	DAIRY							
B.2.1.2	BEEF							
B.2.2	MIXED HERD							
B.2.3	COMMERCIAL HERD							
B.2.4	COMMUNAL GRAZING							
B.2.5	SPECULATOR HERD							
B.2.6	OTHER, PLEASE SPECIFY BENEATH							

(B.3) CATTLE BREED (Please mark with an X)

B.3.1	FRESIAN	
B.3.2	JERSEY	
B.3.3	BONSMARA	
B.3.4	BRAHMAN	
B.3.5	NGUNI	
B.3.6	OTHER, PLEASE SPECIFY BENEATH	

(B.4.1) HERD STRUCTURE ON REFERENCE DATE (see O.3) (Numbers 1 – 6 below refer to farms/plots from B.1.1 and B.1.2)

B.4.1	CATTLE	NO. OF CATTLE ON REFERENCE DATE ON FARMS/PLOTS					
		1	2	3	4	5	6
B.4.1.1	CALVES						
B.4.1.2	HEIFERS						
B.4.1.3	COWS (2-5YEARS)						
B.4.1.4	COWS (5-10YEARS)						
B.4.1.6	COWS (>10YEARS)						
B.4.1.7	BULLS						

(B.4.2) HERD STRUCTURE AT PRESENT (Numbers 1 – 6 below refer to farms/plots from B.1.1 and B.1.2)

B.4.2	CATTLE	NO. OF CATTLE AT PRESENT ON FARMS/PLOTS					
		1	2	3	4	5	6
B.4.2.1	CALVES						
B.4.2.2	HEIFERS						
B.4.2.3	COWS (2-5YEARS)						
B.4.2.4	COWS (5-10YEARS)						
B.4.2.6	COWS (>10YEARS)						
B.4.2.7	BULLS						

(B.4.3) Total Number of OTHER animals on the farm at present:

Sheep	Goats	Pigs	Wildlife-Buffalo	Wildlife-Antelope

(C) MOVEMENT

(C.1) Total No. of cattle introduced in the 12 months before the reference date (see O.3) ☐

(C.2) No. of introductions into herd (WHERE INTERVIEW IS TAKING PLACE) in the 12 months before the reference date (see O.3) (i.e. Number of events when cattle were introduced into the herd) ☐

(C.3) MOVEMENT IN

	SOURCE	No. of Cattle	District	Province
C.3.1.1	Farm – Neighbour			
C.3.1.2	Farm - <20km away			
C.3.1.3	Farm - >20km away			
C.3.2	Auction			
C.3.3	Speculators			
C.3.4.1	Communal grazing – common herd (shared grazing)			
C.3.4.2	Communal herd - <30km away (your cattle do NOT share grazing with this herd)			
C.3.4.3	Communal grazing - >30km away			

(C.4) Were the cattle tested for brucellosis after their arrival? (Y=Yes, N=No, Un=Unknown) ☐

(C.5) Were the cattle vaccinated before arrival onto your farm? (Y=Yes, N=No, Un=Unknown) ☐

(C.5.1) If Y, what vaccine was used?

S19 ☐ RB51 ☐ S19 & RB51 ☐ UNKNOWN ☐

(C.6.1) Were the cattle isolated after arrival? (Y=Yes, N=No, Un=Unknown) ☐

(C.6.2) If Y, for how long ____days

(C.7) Do you check the brucellosis status of the herds you purchase your cattle from before purchase? (Y=Yes, N=No, Un=Unknown) ☐

(C.8) Distance to the nearest infected herd? _____km unknown ☐

(C.9) Were any of your neighbouring farms positive for brucellosis within the last 3 years? (Y=Yes, N=No, Un=Unknown) ☐

(C.10) Do any of your neighbours keep wildlife on their farm? (Y=Yes, N=No, Un=Unknown) ☐

(C. 11) Do any of your workers own their own cattle? (Y=Yes, N=No, Un=Unknown) ☐

(C.11.1) If Yes, please fill the following table:

NAME OF EMPLOYEE	NO. OF CATTLE	GRAZES WITH YOUR HERD? (Y=Yes, N=No, U=Unknown)

(D) MANAGEMENT

(D.1) BREEDING

D.1	Do you... (Y=Yes, N=No, U=Unknown)	
D.1.1	Use bull from another herd?	
D.1.2	Use AI and bull?	
D.1.3	Use AI only?	
D.1.4	Use bull from your own herd?	

(D.2) WHICH OF THE FOLLOWING DID YOU EXPERIENCE IN THE YEAR BEFORE THE REFERENCE DATE? (Please mark with an X)

D.2.1	ABORTIONS IN HERD	
D.2.2	WEAK CALVES	
D.2.3	RETAINED PLACENTAS	
D.2.4	REDUCTION IN NUMBER OF CALVES	
D.2.5	REDUCTION IN MILK YEILD	
D.2.6	REDUCTION IN CONCEPTION RATE	
D.2.7	HYGROMAS IN CATTLE	
D.2.8	REPORTS OF BRUCELLOSIS ON NEIGHBORING FARMS	
D.2.9	MORE THEN 3 MONTHLY CONSECUTIVE POSITIVE MRTS	

(D.3.1) WHICH OF THE FOLLOWING SYMPTOMS WERE EXPERIENCED BY ANY PERSON ON THE FARM/S WITHIN THE LAST YEAR? (Please mark with an X)

D.3.1.1	FEVER	
D.3.1.2	ANOREXIA (no appetite & not eating)	
D.3.1.3	FATIGUE (tiredness & sleepiness)	
D.3.1.4	HEADACHE	
D.3.1.5	DEPRESSION	
D.3.1.6	ANTHRALGIA (i.e. joint pain)	
D.3.1.7	MYALGIA (i.e. muscle pain)	
D.3.1.8	BACK PAIN	
D.3.1.9	EPISODES OF PROFUSE SWEATING	

(D.3.2) If Y, to any of the above symptoms (D.3) how would you classify this person/these persons? (Select most appropriate option by marking with an X. If more than one person per category, state the number of persons showing any of the above symptoms)

Family Members	Employees	Family members of Employees

(D.3.2.1) What sort of animal contact do the people indicated in D.3.2 have with cattle?
(Please specify Y/N/U for each option. Y=Yes, N=No, U=Unknown, NA=Not Applicable)

D.3.2.1.1	CONSUME UNPASTEURISED DAIRY PRODUCTS FROM CATTLE ON FARM	
D.3.2.1.2	VACCINATE COWS WITH BRUCELLOSIS VACCINE	
D.3.2.1.3	HANDLE COWS DURING BIRTH PROCESS	
D.3.2.1.4	OTHER, PLEASE SPECIFY BENEATH	

(D.3.2.2.) Was brucellosis diagnosed in this or any other person on the farm? (Please specify Y=Yes, N=No, U=Unknown)

(D.4) CALVING PRACTICES

D.4 Please answer each of the following (Please specify Y/N/U for each option. Y=Yes, N=No, U=Unknown, NA=Not Applicable)

D.4.1	Do you segregate cows at calving?	
D.4.2	Do dry cows calve together?	
D.4.2.1	If Y, do they calve indoors?	
D.4.2.1.1	If Y, do they calve in individual pens?	
D.4.3	Do you observe each calving?	
D.4.4	Calving practice (management of cows during calving) other than those mentioned above? If Y, please specify beneath	

(D.5) BIOSECURITY

D.5.1 Are your cattle fenced in on your holdings? Y/N/U

D.5.2 How would you describe your cattle handling facilities? (Please mark the most appropriate option with an X)

D.5.2.1	Non-existent	
D.5.2.2	Fair	
D.5.2.3	Good	
D.5.2.4	Excellent	

D.5.3 How do you identify your cattle?

D.5.3.1	Markings only	
D.5.3.2	Ear Tags	
D.5.3.3	Branding	
D.5.3.4	Other	

(E) PREVENTION & CONTROL

(E.1) **Has your herd been vaccinated against brucellosis within the last 3 years?** (Y=Yes, N=No, Un=Unkno ☐

(E.1.1) **If Y, please select the vaccine used:**

☐ S19	☐ RB51	☐ S19 & RB51	☐ UNKNOWN

(E.2) **Have your positive cattle ever been C-branded?** (Y=Yes, N=No, Un=Unknown) ☐

(E.3) **Have you received training in Brucellosis control and management?** (Y=Yes, N=No) ☐

(E.4) **Have your workers received training in Brucellosis control and management within the last 3 years?** (Y=Yes, N=No, Un=Unknown) ☐

(E.4.1) **If Y, who conducted the training?**

THANK YOU FOR YOUR TIME AND INPUT. YOUR CONTRIBUTION IS VERY IMPORTANT IN UNDERSTANDING BRUCELLOSIS IN ORDER TO CONTROL IT.

(F.1) WOULD YOU LIKE FURTHER INFORMATION ON HOW TO CONTROL BRUCELLOSIS IN YOUR HERD? (Y=Yes, N=No) ☐

INTERVIEWER NAME & SURNAME

INTERVIEWER SIGNATURE

DATE OF INTERVIEW

Annexure 2: Animal Ethic approval certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**A case control survey on risk factors for bovine brucellosis in the Eastern Cape province of South Africa**
PROJECT NUMBER	**V010-16**
RESEARCHER/PRINCIPAL INVESTIGATOR	**GT Sandengu**

STUDENT NUMBER (where applicable)	**UP_14447577**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**n/a**	
NUMBER OF ANIMALS	**n/a**	
Approval period to use animals for research/testing purposes		**February 2016-February 2017**
SUPERVISOR	**Prof. D Abernethy**	

KINDLY NOTE:

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

APPROVED	Date **9 March 2016**
CHAIRMAN: UP Animal Ethics Committee	Signature