

**A case control study of risk factors for  
bovine brucellosis in  
KwaZulu-Natal, South Africa**

by

**Nomathamsanqa Nogwebela**

**Submitted in partial fulfilment of the requirements for the degree of  
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in the

**Department of Veterinary Tropical Diseases  
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**Supervisor: Prof. D Abernethy**

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## **Declaration**

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

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**Nomathamsanqa Nogwebela**

**October 2018**

Date

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## **Dedication**

In loving memory of my grandmother who, by educating my father, exposed the whole family to a world of education.

My father who appreciated the value of education enough to educate us and my mother who was always supportive.

My kids; Gugu, Ntsika and Ntinga who had to put up with their mother studying.

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# **Abstract**

## **A case control study of risk factors for bovine brucellosis in KwaZulu-Natal, South Africa**

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Degree: **MSc (Animal/Human/Ecosystem Health)**

Department: **Veterinary Tropical Diseases**

*Brucella abortus*, a major cause of bovine brucellosis, is a facultative intracellular, gram-negative pathogen that infects mammals by replicating inside phagocytic cells of the reticulo-endothelial system and in trophoblastic cells of gravid placentae. As a result, it causes reproductive failure in cattle, with significant economic and animal health implications. It is also an important zoonosis.

Few epidemiological studies of brucellosis have been undertaken in South Africa and consequently, little is known about the drivers of infection. A case-control study was therefore undertaken; 73 case and 102 control herds were recruited from the northern part of KwaZulu-Natal. These district municipalities were chosen because six had experienced cases of brucellosis, either in communal or commercial herds. Livestock owners or employees were interviewed using pre-tested questionnaires. A range of risk factors were identified and assessed including herd characteristics, management factors and knowledge of farm personnel.

The risk factors identified were the herd size, with the odds increasing with increase in herd size up to 26 cattle, the number of and heifers, presence of sheep and goats and, number of sheep and goats, while being government sponsored and knowing the status of neighbouring farm was (protective). The presence of wild ruminants in the neighbouring farm brucellosis clinical signs in cattle and owners receiving training in the control of brucellosis were found to be an indication of risk.



The study provided valuable information on drivers for brucellosis and will assist owners and programme managers in the control of bovine brucellosis and thus contribute to the on-going eradication programs in KwaZulu-Natal.

## Introduction

*Brucella abortus*, the major cause of bovine brucellosis, is a facultative intracellular pathogen that infects mainly cattle but also other livestock species. *Brucella* organisms are gram negative, coccobacilli within the class Alphaproteobacteria (Olsen & Tatum, 2010). The parasite replicates inside the phagocytic cells of the reticulo-endothelial system and, in pregnant animals, inside trophoblastic cells of the placenta. They are characterised by being extremely adapted to the host (Moreno, 2014). Bovine brucellosis can sometimes be caused by *Brucella melitensis* less so by *Brucella suis* (OIE Manual, 2004).

*Brucella abortus* infection has significant animal and public health implications as it causes reproductive failure in animals (Poester et al., 2010). The consequences are adverse economic effects on the livestock economy and the disease is an important zoonosis. Few epidemiological studies have been undertaken in South Africa, which has experienced a rising prevalence for some years. KwaZulu-Natal has recently experienced bovine brucellosis outbreaks in commercial herds in the Amajuba-UMzinyathi district municipalities (personal experience of the Researcher as one of the two State Veterinarians in charge of the two Municipalities). This was followed by a province-wide survey of communal herds, which revealed a substantial problem in the north eastern part of the province. Objectives of the study were therefore to:

- (i) optimize control measures to be implemented
- (ii) inform the education and extension strategy that will be given to control scheme managers and farmers
- (iii) Direct policymakers to develop and implement control strategies
- (iv) sensitize human health officials
- (v) public health in terms of milk hygiene and biosecurity
- (vi) help politicians to direct funding priorities

## Literature review

### Aetiology

The major causative agents of bovine brucellosis are the gram-negative coccobacilli, *B. abortus* (major cause, in cattle) less so by *B. mellitensis* (Solera et al., 1997; Bengis et al., 2004) and very rarely *B. suis* (Bishop et al., 1994, Godfroid et al., 2011, Moreno, 2014). The *B. mellitensis* was reported to be of most important zoonotic implications (Gomo et al., 2012) *B. ovis* may have subjected to selection processes when sheep were domesticated, but it is not known to be a rough strain which limits the ability to cause infections to other hosts (Moreno 2014). Brucella has several biotypes (subspecies), *B. abortus* (biovars 1, 2 and 4), vaccine strains *B. abortus* RB51 and S19, *B. mellitensis* (biovars 1, 2 and 3), vaccine *B. melitensis* rev1 and *B. suis* (biovar1) as isolated by AMOS multiplex PCR assays (Gomo et al., 2012, Bricker & Halling 1994), but due to unavailability of phage tests with *B. abortus* only two biovars could be confirmed using microbiology tests (Alton et al., 1988).

### Pathogenesis

Brucellosis infection occurs when *Brucella* organisms invade the host via cut skin, conjunctivae, respiratory mucosae or the gastrointestinal tract, following ingestion of contaminated material, the latter being more common (Neta et al., 2010). The bacteria, once inside the body, are taken up in local lymph nodes where they replicate intracellularly in phagocytes and, unless eliminated, spread to cause systemic infection, colonization of the pregnant uterus, male genital organs and mammary glands (Neta et al., 2010). Reasons why the organisms persist, multiply inside the phagocytic cells as well as the interaction with their hosts are not clear, despite substantial research (Seleem et al., 2008). Inability to express classical virulence factors, has been mentioned as one of the survival factors for *Brucella* spp. therefore introductions of infection into clean herds should be prevented (Seleem et al., 2008).

The success of the *B. abortus* organism in cellular invasion is based on its ability to adapt to the intra-cellular environment, avoid activation of the immune system and disturbing trafficking inside the cell (Seleem et al., 2008). This survival is further facilitated by the genes that enable the adaptation to deprivation of oxygen inside the macrophages (Seleem et al., 2008). Innate immunity (which also includes natural resistance by the host), plays a key role during *B. abortus* infection since it reduces the infective dose and may stimulate the development of a protective adaptive immunity (Neta et al., 2010; Adams & Templeton, 1998). The resistant phenotype is associated with the nature of bovine macrophages to prevent intracellular growth of *B. abortus* (Campbell & Adams; 1992; Campbell et al., 1994; Qureshi et al., 1996). It has been demonstrated that the Bovine brucellosis pathogen cannot induce a significant pro-inflammatory reaction during infection in mice and has a low immune stimulatory activity and toxicity for host cells (Barquero-Calvo et al., 2007). This ability to inhibit, avoid or delay immune response may be a strategy of the organism to cause persistent infection in the host (Neta et al., 2010). Under field conditions the adaptive strategy of *B. abortus* organism is the ability to align itself with the metabolism of the host and feeds on the different nutrients in the host (Brown et al., 2008; Lamontagne et al., 2010).

*Brucella* organisms replicate massively in the placental trophoblasts during gestation, disrupting the pregnant uterus and inducing abortion (Godfroid et al., 2011). Aborted foetuses are generally fresh with minimum autolysis (Olsen & Tatum, 2010), but under field conditions the aborted cows show no apparent clinical signs leading to late discovery of the case of abortion.

According to Olsen and Tatum (2010), antibodies have a small role to play in the long-term protection against *B. abortus*, the major protection is through adaptive immune response, particularly cell mediated (Olsen & Tatum, 2010, Olsen and Tatum, 2016). *Brucella abortus* infection characterizes itself by the ability of the body to activate the innate and adaptive immunity. This then leads to initiation of inflammatory response that favours the T-lymphocytes to differentiate towards T-helper 1 profile (Baldwin, 2002). It has furthermore been shown that cultured macrophages from *Bos Indicus* were significantly more potent in controlling replication inside the cells of *B. abortus*,

suggesting that this cattle breed is likelier to have a higher resistance to bovine brucellosis than *Bos Taurus* breeds (Macedo et al., 2013). The disease can be transmitted vertically through contaminated milk (Neta et al., 2010; Olsen & Tatum; 2010).

## **Clinical signs**

Aborted fetuses are often not available under field conditions due to severe autolysis which prevents useful pathological examination (Neta et al., 2010).

In female cattle, infection is associated with late trimester abortion, retained placentas, birth of weak calves, reduction in milk production, increased somatic cell count and reduced reproductive success (Neta et al., 2010). Aborted fetuses are generally fresh with minimal autolysis; these fetuses become the most important source of infection. Infected males are characterised by epididymitis, orchitis, unilateral and/or bilateral atrophy of the testicles, abnormality of sperms and infertility (Megid et al., 2010). In the African herds hygromas are seen as supportive evidence of the presence of brucellosis in males (Gomo et al., 2012), especially because animals are kept longer enough to develop chronicity (McDermott and Arimi, 2002)

## **Distribution**

Bovine brucellosis has a worldwide distribution; some countries have eradicated it, some are in the stages of eradication while in most of the third world countries, it is still a huge problem.

**Table 1** Brucellosis eradication/control schemes in some parts of the world

Programme	Year	Area/Region	Reference
Diagnosis of <i>Brucella</i>	1887	Malta, UK	Poester et al., 2010
<i>Brucella abortus</i> control programmes	1934	USA	Olsen & Tatum, 2010
New Zealand Brucellosis eradication scheme	1979	New Zealand	Cordes & Carter, 1979
Bovine brucellosis to a national control programme in breeding herd	1968	Italy	Dalla Pozza et al., 1997
Herd prevalence in intensively farmed cattle	1990	USA	Hesterberg et al., 2008
Initiation of control measures	1964	Northern Ireland	Abernethy et al., 2011
First report	1939	Egypt	Holt et al., 2011
First recognition	1942	India	Renukaradhaya et al., 2002
Bovine brucellosis reporting in Southern Africa	1913	Southern Africa ( <i>Zimbabwe, Botswana</i> )	Bevan, 1931
	2009	<i>B. abortus</i> report in Zimbabwe and endemicity of Brucellosis in Southern Africa	Gomo et al.,2012
	2009	<i>B. mellitensis</i> biovar isolated in Zimbabwe	Matope et al.,2009

Table 1 illustrates some brucellosis control schemes in existence in various parts of the world, including Africa, since its discovery early 19<sup>th</sup> century (only showing some countries, selected randomly).

## Epidemiology

### South Africa: current situation/control strategies/KwaZulu-Natal

Studies within South Africa have estimated the prevalence of bovine brucellosis to range as shown in the table 2 below over years.

**Table 2** Results of a study of sero-prevalence of Bovine brucellosis in KwaZulu-Natal in abattoirs, communal dip-tanks and as represented by diseases related abortions

Survey/Sample Type	Year	Area/Region	Animal Prev. (%)	Reference
Cattle in abattoirs	1982	KwaZulu-Natal	1.5	Bishop (1984)
Cattle at dip tanks	2001	KwaZulu-Natal	1.45	Hesterberg et al. (2008)
Abortions due	2011	Gauteng Province	3.8	Njiro et al. (2011)
Communal cattle	2014	KwaZulu-Natal	1.3	Chisi (2014)

In South Africa, 290-460 bovine brucellosis outbreaks were reported annually to the OIE between 1996 and 2004 (OIE, 2006) while the herd prevalence in the intensively farmed areas was 14.7% in 1990, resulting in R300 million in losses annually (Hesterberg et al., 2008). South Africa started with a variety of control efforts, including vaccination, testing, movement control, and stamping out activities. Zimbabwe also introduced targeted programs aimed at specific production groups; however, outside southern Africa, no attempt has been documented to systematically eradicate or control the disease (Demenech et al., 1980).

The north eastern part of KwaZulu-Natal (KZN) province is predominantly beef farming, the south eastern area largely dairy while mixed dairy and beef farming occurs in the west (Hesterberg et al., 2008). Commercial dairy cattle are tested monthly by milk ring testing and the farmers generally follow a vaccination and control strategy. Testing in commercial beef herds is done every five years. This, however, these results in the brucellosis status being uncertain and poorly controlled (Hesterberg et al., 2008).

Various studies done in most of Southern African countries showed more prevalence associated with commercial than communal farms (Matope et al., 2010, Gomo et al., 2012) but in the study done in KwaZulu-Natal the highest number of seropositive animals was found to be at the North-eastern part of the province in UMkhanyakude district, communal cattle (Hesterberg et al., 2008), same prevalence results were confirmed by Chisi et al., 2014. The study by Hesterberg et al 2008 showed that the knowledge about bovine brucellosis was very low or non-existent amongst the dip tank cattle owners in the rural communities as well as the commercial farmers. It further

showed that there was also minimum knowledge on the effect of the diseases on people and cattle by the traditional farmers. The study therefore concluded by suggesting prioritization of information and implementation of diseases control measures. It was found that besides the public being ignorant of these problem diseases, there was also a lack of awareness even with the human health officials resulting in the lack of prevention and management by the health officials, that hindsight could possibly encourage the transmission of the infection (Hesterberg et al., 2008).



## Materials and methods

### Study design

A case control study design was used. A case control study is the best approach to study rare diseases or outcomes as it does not need to be based on the prevalence of the disease. The case control studies are of practical value for the rapid production of results making them ideal type of study as a preliminary disease investigation and are cost efficient. The population at risk was all *Brucella abortus*-susceptible cattle in the province of KwaZulu-Natal, in both rural communal and commercial herds. The study population was all the farms/dip-tanks that were sampled by Chisi et al. (2014) in 'Study of KwaZulu-Natal Provincial Sero-Prevalence Survey in Communal Cattle', except that commercial cattle tested during the same period were also included in the study, (excluding the Municipalities in the Southern part of the province, who had a low prevalence). Communal herds are herds where there are two or more animals belonging to different owners but kept together under one management system thus representing one epidemiologic unit, while a commercial herd usually belongs to the same owner, raised separately from other herds and not linked with another herd (EU, 2009).

Case farms were defined as those with at least one *Brucella*-positive animal, confirmed by bacteriological culture or two or more animals that were positive to the SAT and CFT and where vaccination was unlikely to be the cause. In the case of a commercial farm, two or more positive animals or a bacterial culture positive in the same farm resulted in the whole farm defined as positive. Controls were defined as farms or herds/dip tanks within the same State Veterinary area as the case herd, with no clinical symptoms of bovine brucellosis and where all cattle tested negative within six months of a case herd. For every positive case there were ideally two control cases selected. Accordingly, control herds were recruited from the same areas of the province as cases, except for UMkhanyakude (Jozini area) due to resource constraints and responsibilities related to Foot and Mouth Disease control in the area.

A sample size of 176 herds was selected, based on prevalence of 1.3%, 95% CI; 1.08%-1.53%) Chisi et al. (2014). One hundred and fifty-four (88%) of the interviewees were herd owners, 8.6% were employees while 3% were managers.

A questionnaire was prepared by a group of University of Pretoria Master's students, of which the researcher was a part of, who were to undertake similar studies in selected Provinces of South Africa. The questionnaire was trial-tested on three farms in KwaZulu-Natal by the researcher, before being released to KwaZulu-Natal Animal Health Technicians (AHT's). These AHT's later undertook the contact interviews with participating farmers after a training workshop that was held by the researcher prior to their commencement of the field work. Consent was obtained from each participating farmer. The questionnaires were returned to the State Veterinarian's office of origin where the responses were recorded on a Microsoft Access database. Quality control checks were done by the Researcher by checking every tenth response to see if all details were correct.

Data were stored and collated in an Access 2013 database and analysed in Excel 2013 and SPSS (IBM, Version 25). Unadjusted odds ratios were calculated from a univariate analysis and adjusted estimates from a binomial logistic regression. Upon completion of the univariate analysis, all variables with a probability value  $P < 0.25$  could 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. 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

### General

One hundred and seventy-five herds of cattle, consisting of 73 which were cases and 102 controls were recruited for the study. Herds were recruited from seven municipalities with a median of 18 herds per municipality (range 8-60). The reference dates for the study were from 1 October 2013 to 31 October 2015. The results show that while some risk factors such as, being a government sponsored farm, presence of sheep and goats farmed together with cattle, as well as their presence and their increasing number, knowing the brucella status of neighbouring farm, noticing brucellosis clinical signs in cattle and owners receiving training in brucellosis are protective it looks like the increase in herd size, the numbers and proportion of cows and heifers and presence of wild ruminants appear to be more important risk factors.

### Herd Size

The median herd size at interview was 18 cattle (range 1-627) with case herds being larger than control herds (median in case herds = 20.5; Tukey's hinges = 12.5 and 31.0 respectively and 16.0 in control herds 16.0; Tukey's hinges = 9.0, 16 and 32 respectively). The median number of cows was also higher in the case herds compared to the control herds (10 versus 9). There was an increased risk of the disease with increasing herd size, with larger herds having approximately twice the risk of a breakdown when compared to the smallest stratum (1-10 cattle), see table 3. However, there was no linear increase in risk with increasing herd size. There was also no significant difference when the numbers of strata were increased from three to four. There was also an increased risk associated with an increasing number of cows and heifers: herds with more female cattle had an approximately 2.5 increase in risk (Table 3), compared to the smallest stratum of herds. However, the proportion of female cattle in the herd was not significantly associated with an increase in risk (Table 3).

One hundred and sixty-six (94%) herds utilised only one holding. For 160 herds (91.4%), where the origin of the herd was declared, 16 (10%) were government sponsored of which one was a case herd (OR = 0.079; 95% CI = 0.010 - 0.61; p-value = 0.029).

One hundred and sixty-three (93.1%) herds were classified as beef herds, three (1.7%) as dairy and nine (5.2 %) were mixed. One hundred and twenty-four herds (70.9%) also contained small ruminants (median number = 14; range 2-1200). There was no correlation between the number of cattle and the number of small ruminants in a herd (Pearson's Correlation = 0.046; p = 0.547).

Few herds (n = 20 or 11.4%) acquired cattle in the previous year, nine case herds and eleven control herds.

**Table 3** Univariate analysis of the risk factors for bovine brucellosis

Variable		Odds Ratio	95% C.I.	ChiSq	p-value
Number of cattle	1-12 (n = 59)	1	-	-	-
	13-26 (n = 58)	2.126	0.998-4.530	3.87	0.05
	> 26 (n = 58)	1.984	0.930-4.230	3.181	0.075
Number of cows & heifers	1-8 (n = 52)	1	-	-	-
	9-17 (n = 61)	2.627	1.190-5.800	5.849	0.016
	> 17 (n = 62)	2.385	1.082-5.257	4.744	0.029
Proportion of cows & heifers	< 0.64 (n = 58)	1	-	-	-
	0.64-0.74 (n = 60)	1.159	0.559-2.403	0.158	0.691
	> 0.74 (n = 57)	0.89	0.422-1.879	0.093	0.761
Government-sponsored herd (Y/N)		0.079	0.01 - 0.612	-	0.002*
Additional farms (Y/N)		0.787	0.222 -2.793	-	0.764*
Beef v dairy/mixed		2.258	0.590-8.649	-	0.364*
Sheep or goats present (Y/N)		0.155	0.075-0.320	28.15	< 0.001
Number of sheep & goats	0 (n = 52)	1	-	-	-
	1-14 (n = 52)	0.098	0.041-0.232	31.06	< 0.001
	> 14 (n = 64)	0.208	0.093-0.465	15.44	< 0.001
Inward movement of cattle		1.163	0.456-2.970	0.1	0.752
Know status of neighbour (Y/N)		0.196	0.098-0.391	23.08	< 0.001
Wild ruminants on neighbouring farm		2.511	1.097-5.749	4.949	0.026
Brucellosis clinical signs in cattle (Y/N)		0.42	0.227-0.777	7.767	0.006
Brucellosis symptoms in people (Y/N)		0.888	0.410-1.925	0.09	0.764
Increase in biosecurity for calving (Y/N)		1.486	0.806-2.738	1.619	0.203
Cattle fenced in (Y/N)		1.19	0.584-2.424	0.23	0.631
Owners received training in brucellosis control (Y/N)		0.262	0.102-0.674	8.494	0.004
Workers received training in brucellosis control (Y/N)		0.864	0.271-2.756	0.061	0.805

\*Fishers Exact Test

## Presence of sheep and goats

The presence of small ruminants was significantly protective, and this was consistent across strata of increasing small ruminant numbers (Table 3) and increasing cattle numbers (Table 3).

**Table 4** Cochran-Mantel-Haenszel Test for 2x2xK tables of three strata of risk with increase in herd size

	<b>Odds Ratio</b>	<b>Lower</b>	<b>Upper</b>	<b>ChiSqd</b>	<b>p-Value</b>
1-12 Cattle	0.257	0.075	0.885	4.943	0.026
13-26 Cattle	0.154	0.042	0.557	9.097	0.003
> 26 Cattle	0.086	0.021	0.352	14.192	< 0.001
<b>All Strata</b>	<b>0.155</b>	<b>0.075</b>	<b>0.32</b>	<b>28.146</b>	<b>&lt; 0.001</b>

## Spatial factors

More than ninety one per cent (91.4%; 160/175) of farmers interviewed did not know the location of the nearest positive (breakdown) herd. Of the 15 interviewees who did know, seven were case herds and all stated the nearest herd was within 50 km. For the eight control herds, seven advised the nearest infected herd was greater than 50 km away. Similarly, only 41.7% of interviewees knew if their neighbours had experienced a brucellosis outbreak within the last three years; knowing the neighbour's status was protective (OR = 0.196 - Table 3) and for those interviewees that did know the status, the odds of a case herd having a positive neighbour was 27 times that of the control herds ( $p < 0.001$ ).

## **Workers owning cattle**

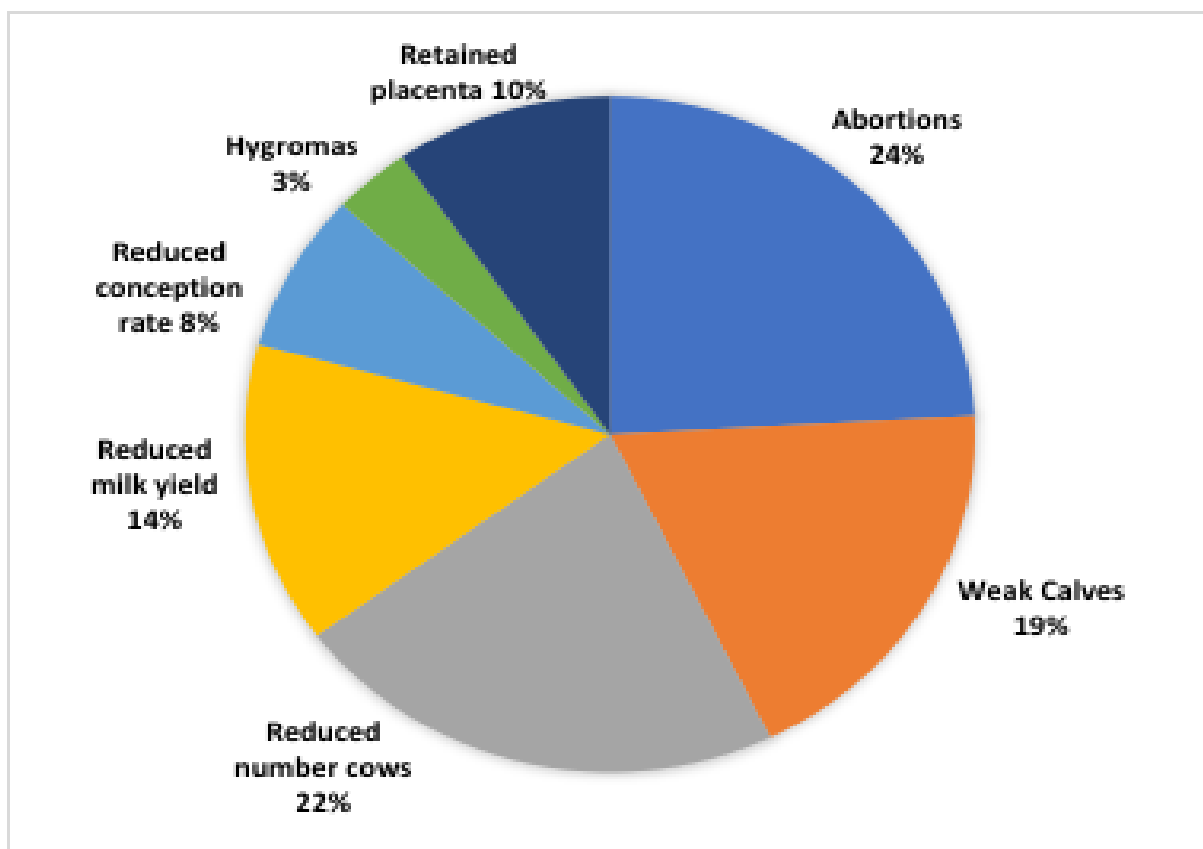
More than thirty per cent (30.3%; 53/175) of interviewees did not know if workers owned their own cattle (thus not included in univariate table). There was no association, for those farmers who did know, between workers' ownership and the risk of brucellosis (OR = 1.267; 95% C.I.: 0.655-2.454; p = 0.482).

## **Breeding methods**

Only five farmers (2.86%) used AI, with or without a bull. For those farmers who used a bull, there was no significant association between those who used their own bull and those who used the neighbour's bull (OR = 1.477; 95% C.I.: 0.768-2.841;  $\chi^2 = 1.374$ ; p = 0.241)

## **Clinical signs**

Of the 96 interviewees, figure 1, (54.9%) who reported brucellosis-associated signs, the most common was abortions (24%). Of those farmers who had observed symptoms, 71.9% reported only one symptom, the remainder (14.3% of all interviewed) reported two to five symptoms, as demonstrated by the pie chart below.



**Figure 1** Pie chart of distribution of clinical signs reported by interviewees

Observation of any *Brucella*-associated clinical signs in the herd was significantly protective (Table 3), however, for those farmers who reported these clinical signs to the interviewer (n = 96), having multiple symptoms was associated with a statistically significant increase in risk.

## Symptoms in people

A wide range of symptoms were reported in people, but the prevalence was very low: 'Fever' was the most commonly reported (7.4%) and there was no significant association between having any symptoms and brucellosis in the herd (Table 3). There was also no difference between the proportion of family members or workers who reported symptoms (56.7% v 43.3% respectively;  $\chi^2 = 1.059$ ;  $p = 0.303$ ).



## Management practices

Forty four per cent (44%; 77/175) of those interviewed undertook no interventions to improve biosecurity around calving. There was no statistically significant association between taking any initiative and the risk of brucellosis (Table 3) and this was observed across all strata of herd size (Cochran-Mantel-Haenszel Test (table 4); data not shown;  $p > 0.05$  across all strata). The results also showed that almost all (95.6%; 169/175) of farmers interviewed used some form of identification, with the most common being branding. There was no significant difference between cases and controls in respect of the level or type of identification used.

Overall, 71.4% of interviewees knew the brucellosis vaccination history of their herd but only 44.4% advised their herds were vaccinated. A significantly higher proportion of control herds knew their status compared to case herds (77.5% v 63.0%;  $\chi^2 = 4.361$ ;  $p = 0.037$ ). Of the farmers who knew their status, 87% of case farms and 48.1% of controls farms were reported to have been vaccinated within the previous three years. Of the 30.8% who were vaccinating their cattle, only 13.7% of all interviewees vaccinated their cattle with S19, either alone or in conjunction with RB51 and only four case herds (5.5%) had their cattle C-branded.

## Training

More than eighteen percent (18.3%) of interviewees had received training in brucellosis control and this was highly protective (OR = 0.262; Table 3). Workers on 7.4% of farms had received brucellosis training in the last three years but although this was also protective (OR = 0.864; Table 3), it was not statistically significant.

## Multivariate analysis

In the multivariate analysis (table 5), only herd size remained as a statistically significant risk factor.

**Table 5** Multivariate analysis of strata of herd size as a risk factor

	<b>Variable</b>	<b>Odds Ratio</b>	<b>95% C.I.</b>	<b>ChiSq</b>	<b>p-value</b>
Herd Size	1-10 (n = 47)	1	-	-	-
	11-18 (n = 42)	2.409	0.986-5.890	3.794	0.074
	19-32 (n = 43)	4.050	1.659-9.891	9.862	0.003
	> 32 (n = 43)	1.907	0.778-4.674	2.016	0.180

## Discussion

It was the first time that the risk factors for bovine brucellosis were explored in KwaZulu-Natal, in a case control study. There was a good co-operation between the farmers and the interviewers (Animal Health Technicians). The challenges with the study related to the farmers being asked exposures that occurred six or more months previously (retrospective study) leading to recall bias. The conclusions from the study will be used to justify the control measures to be applied later. This was facilitated by the training the staff received at the workshop which assured us of consistent approach to questioning.

Our study has shown a significant association between increasing herd size and the risk of brucellosis, but this linear relationship becomes apparent when the herd size is broken into four strata. This was the only variable that was significant in the multivariate analysis. Significantly, this finding was observed despite most herds being recruited from the communal sector, where herds are normally smaller than the commercial sector. In KwaZulu-Natal, farmers fall into different strata of farming. There are those who are doing sustenance farming, as a group of communally grazed cattle with fewer herds of cattle. These cattle are communal dip-tank based and grazing together as a single herd, freely interacting with no fences demarcating different farming systems only separating when they are herded to different kraals at night time (extensive system). There is also a stratum of farmers who show a tendency towards intensive farming for commercial purposes. These have larger herd sizes than the subsistence group, but the herd size varies per individual farmer, but generally may be above 32. The top stratum is commercial farmers with more intensive cattle farming and bigger herd sizes. The top stratum is likely to be more observant of basic biosecurity than the strata below them and likely to have better control / management on the movement of cattle. The combination of lack in biosecurity in the herd, lack of control of animal movement is associated with the epidemiology of contagious diseases of which bovine brucellosis is one of the diseases of concern as observed by Anka et al., (2014).

This study also suggests and concurs with literature (Crawford et al., 1990; Salman & Meyer, 1984) that farming with a bigger herd, in the absence of proper biosecurity and separation of the herd, is a risk factor for introducing and sustaining the disease in the herd. The increase in herd size (resulting in bigger herds), according to Olsen and Tatum (2010) corresponds with the increase in direct and indirect mucosal contact through air from abortion fluids and aborted foetuses. This then, increases the risk of infection and the difficulty of cleaning the herds from the bovine brucellosis. The efforts towards bovine brucellosis management should be concentrated on encouraging the farmer to increase their management and control as the herd gets to be above 19 in size. This effort will decrease the risk of the farm contracting the disease and having to deal with its chronicity. The ideal advice is vaccination of cattle irrespective of the herd size, though with increased number of cattle this should be accompanied with conscious management of prevention and control strategies instead of waiting for a bigger herd before the implementation of proper biosecurity measures. The risk with increase in herd size is also associated with the increase in the number of cows and heifers (female herd), with the odds of a herd with > 9 female animals increasing the risk of bovine brucellosis by 2-3 times as found by our study. As the number of female cattle was closely correlated with overall herd size, this variable was not included in the multivariate analysis. Nevertheless, the study agrees with the general understanding that the surveillance and control activities are always targeted towards the female herd together with the fact that in any herd the proportion of the female herd is higher than males. The proportion of the cow herd to heifers did not show any significance despite the expectation. Mangen et al. (2008), in their study, mentioned 2.5 times higher odds when comparing the cow population and the population of cattle (assuming the population of cattle to be inclusive of heifers, cows, steers and male cattle). In KZN, heifers are not tested until after they calved (or after three years if they failed to be in calf), ideally female herds will have cows in majority. The significance of herd size and female herd size emphasizes the importance of vaccination -not only of cows but also in heifers, to avoid disease chronicity in the cow herd as the brucellosis policy suggests.

The presence of sheep/goats in the herd was shown to be protective: herds with small ruminants having approximately one quarter of the risk of cattle-only herds. This finding is consistent with a parallel study in the Eastern Cape (G. Sandengu, pers. comm.). Such findings contrast with the literature: Anka et al. (2014) reported that farms with sheep/goats are five times more at risk than cattle-only farms while other authors report similar findings e.g. Dalla-Poza et al. (1997). These authors also report that mixing of sheep and goats at the water resources in communal dip-tanks can be a risk factor for the disease.

Bovine brucellosis has a complex epidemiology (Crawford et al., 1990; Caporale et al., 2009) such that other criteria than the test is required for the successful control and eradication of the diseases. This also means that S19 and Rev 1 (implying the importance of small stock considerations) vaccines must be considered as the corner stones of the control and eradication programmes of bovine brucellosis.

It is not clear why small ruminants are protective in these studies, but it might reflect difference in management styles between such mixed herds and cattle-only unit. Further studies are required to investigate this further, but it is advisable that farms with increasing number of sheep and goats should give special considerations to stricter controlling measures or to separate the two enterprises in space or time to be able to manage or prevent introductions/maintenance/eradication of bovine brucellosis especially where there is greater risk of *B.mellitensis*.

The study found no statistical significance in either farming with beef or dairy. This finding concurs with the finding by Anka et al. (2014) in Malaysia that there was no increase in risk in either of the enterprises. However Gomo et al., 2012, and Matope et al., 2010 found involvement biased towards commercial farming enterprises. Similarly, the number of farms was not significant suggesting that either information on the different activities lacking (e.g. association between herd size and number of premises) or animal movements between premises or to other premises is more important.

Government sponsored farms had a significantly lower risk (OR = 0.79; P = 0.002) than non-government sponsored herds. No previous studies have been undertaken and therefore no comparative data exist, but it might reflect better biosecurity, the recent acquisition of these farms or that, to buy the farm, a declaration of disease freedom is required.

The study could not associate the movement of cattle with increased risk of bovine brucellosis. This reflected the low number of herds in which movement was reported and further investigation is required to assess this reportedly important risk factor. Hesterberg et al. (2008) warned that purchasing infected livestock combined with the long testing intervals as is seen in KwaZulu-Natal communal or beef farms is a risk factor in maintenance of bovine brucellosis in cattle herds. Holt et al. (2011) even argued that people of Egypt would sell their infected cattle anywhere, thereby increasing the risk of infection to uninfected farms or regions. Hence Smits (2013) concluded that the success of control of the disease should be based on the knowledge of the epidemiology of the disease (buying in is a risk factor included in the epidemiology). Matope et al. (2010) argued that free movement and intermingling of extensively managed herds of cattle is a risk factor in the bovine brucellosis which is the same thing happening in herds that keep on moving cattle. Reasons for this apparent lack of support by our study could be that the farmers did not consider movement within the dip tank level a 'movement', or perhaps that such cattle were not seen to be 'purchased' or 'acquired'. Communal people are known to move their cattle a lot, for lobola payments or assisting those who do not have cattle by donating animals. Thus, although the study failed to demonstrate the importance of movement, farmers should be encouraged to move the animals responsibly through movement permits or with accompanying bovine brucellosis free declarations. This responsible movement of cattle should be encouraged even in communal dip tanks where the movement could be cultural rather than for financial reasons.

Knowing the brucellosis status of a neighbouring farm was protective at the univariate level, which might reflect that farmers who are more concerned about the biosecurity of their farms are on the look out of the possible risk factors to their farms. It makes sense to assume that those farmers act responsibly towards cattle ownership and

biosecurity, including associating with farms of known disease status. Hesterberg et al. (2008) showed that one of the major risk factors in bovine brucellosis transmission is the proximity of the infected herds to each other, sharing of the pastures and together with the lack of knowledge on the disease are the factors that encourage transmission to non-infected herds. Smits (2013) even argues that the increase in community participation can be used effectively to reduce the spread of the disease.

The study agrees with the literature that the lack of knowledge is a risk for propagation of bovine brucellosis. The disease control activities must be focused on imparting knowledge about the disease, together with making the neighbouring farm status known to improve surveillance measures. It has been shown that a lack of knowledge gives a false sense of security. The farmers with no disease concern issues do not bother to know the status of the neighbouring farm. Responsible farming means to be more proactive in taking measures to prevent incidences of diseases. Amongst the responsible actions is cooperation with the State Veterinary activities on the disease management. The fact that the owners of positive farms suddenly know which other farms are positive means that it was a stimulus which leads to each seeking and sharing knowledge and therefore arming themselves about the status and expectations of the diseases control measures.

Farms neighbouring those with wild ruminants' owned farms were three times more likely to go down with brucellosis infection in their herds than those who are not, which is consistent with the findings by Anka et al. (2014) who reported a 42-fold increase in risk when cattle were farmed with wild-life species. These authors further argued that the presence of wild life in brucellosis endemic areas becomes a concern because wild life could be infected as a spill over from infected cattle, and thereby becoming a reservoir of infection for cattle. Godfroid et al. (2011) warned of the role of an expanding wild life reservoir, the control of the livestock-wildlife interface and the role it was playing in the complication of bovine brucellosis control. They emphasized the importance of surveillance of cattle and the wildlife together. Developing control strategies that involved the livestock-wildlife interface was encouraged by Dalla-Pozza et al. (1997) and Smits (2013). Olsen (2010) argued that the control in wild life will be more challenging if the same strategy to be used is the same as in controlling of the

disease in cattle. Thus, from this study, farmers involved in this interaction should be encouraged to work out the ways to co-exist with these different enterprises. Included in their agreement should be finding a strategic way to deal with the challenge with special emphasis on separation of the two enterprises (in space or time) especially during critical times of drought and calving. The surveillance activities, with the intention at control at these livestock-wildlife interfaces should be encouraged.

Discovering clinical signs of bovine brucellosis was found to be protective. It might be that farmers who notice symptoms become pre-armed, even if disease is not present, and may therefore take precautionary measures. Pie chart 1 above shows that the most noticed clinical sign is abortion (24%), reduced number of calves (22%) weak calves (19%), reduced milk yield (14%) and the other signs, such as retained placentas, reduced conception rate and hygromas are noticed to a lesser extent. The lower proportion of observation of the abortion, weak calves and reduced milk yields could be attributed to the fact that KwaZulu-Natal communal farmers practise an extensive farming, reducing the chances of observation of these signs when opposed to farms that are intensive. Like Anka et al. (2014) mentioned in countries such as Malaysia, signs like abortions and still births are seldom noticed or reported. This non-reporting gets complicated by unplanned breeding in these farming systems, becoming a huge risk factor.

In the experience of this researcher, some farmers seldom practise observing the animals for these signs. There is therefore a need to have the farmers educated on these issues so that they know the early warning signs of the disease and take precaution. These could then be followed by investigations and diagnosis which will help reduce the spread of the disease to naïve cattle. This will also help the farmer to put in control measures such as separation of the infected herd, culling and vaccination according to need. The early detection will help reduction of losses and prevent the spread of the disease during the time when the cows are excreting the pathogen. The farmers need to appreciate that brucellosis as a disease may not be noticed and therefore the abortions/at calving time are the critical times for the first clinical sign to lead to suspicion and finally to a diagnosis. The study showed that 44% of the farmers did not improve their security measures around calving time irrespective of the size of



the farm (different strata). Abortions occur in the last trimester of gestation and the fact that abortions, as one of the risk factors (indicators) for the disease is associated with (poor) husbandry practices. According to Corner (1983), this timing of abortions explains the between herds transmission, within herds and a long time from discovery to sorting out breaks. The knowledge of the timing of abortion could be used to determine the most significant times of controlling, segregation, observation at calving and culling of aborting/ test positive animals. This knowledge would play a role in the control of bovine brucellosis, transmission between herds, within herd prevalence and resolution of outbreaks (Abernethy et al., 2011; Samartino & Enright, 1993). McDermott and Arimi, (2002) argued that the major economic losses are those associated with abortions, impaired fertility, including milk production and 10% loss in calf crop in brucellosis positive dams. According to Olsen and Tatum (2010) control programs, when implemented assist in the prevention of economic losses. These may be expensive to implement, require good record keeping and infrastructure which is the reason why bovine brucellosis programs in some countries are aimed at control rather than eradication.

The study found that the farmers fall short at managing biosecurity of the cattle around calving time, due to management systems which do not allow segregation of last trimester pregnant cows. In cases such as these, the farmer may not be able to tell when the cow is due to calve; if it aborts he may not know where the aborted foetus is. No information can be provided to facilitate diagnosis of abortion. Farmers, whether commercial or subsistence/communal should be able to organise their farming conditions such that calving occurs in a well-protected clean environment. That way they could be able to notice stillbirths or weak calves being born, which cows are poor in milking in the case of beef cattle and dairy cows will be seen in the milking parlour if they have reduced milk yield. Management during calving period help prevent economic losses that happen around this time. Attention should also be given to stop unnecessary contamination of the farming area. It has been proven that good bovine brucellosis management depends on good control measures, which include biosecurity, isolation of suspected, calving or aborting animals, vaccination, branding and good record keeping. This will help the farmer to identify problem animals in time and to remove them from the herd.

According to the study, farmers appeared to be knowledgeable about cattle vaccination such that they could tell whether their cattle were vaccinated or not, although fewer than 50% vaccinated their cattle. The RB51 appeared to have been the vaccine of choice, though a fewer of them reported using both S19 and RB51 or some S19 alone. Very few of the positive farms seemed to have their cattle C-branded. Government officials should be encouraged to C-brand positive animals to reduce the cases where they are taken to auction for sale.

Farmer education initiatives should include data management around cattle farming which will involve the farmer knowing which part during the gestation when abortions are expected should they be there, encouraging farmers to get rid of *Brucella* reactive and or aborting animals. If the animals are not kept under appropriate biosecurity measures, there is no way of knowing which animals are doing better and which ones are worst. Cattle identification and vaccination should be cornerstones of effective cattle management and that should include branding of test positive animals.

Providing training to farmers was statistically significant and protective at the univariate level though that was not the case with training of workers. This can be explained by the fact that the decision makers (in the farm) are the farm owners; therefore, arming them achieved better results. Smits (2013) argued that the lack of data was one of the significant risk factors of concern on the part of practices of farmers and livestock owners. These are the factors that support the epidemiology, knowledge and attitudes and lack of these is a drawback in the disease control. Armed with these useful data can be used productively by farmers to influence policymaking and financial priorities, as suggested by Smits (2013). The inclusion of community participation is the backbone of disease control. There is value in farmers knowing the strategic times to put their energy in the control of this disease, because other than in late gestation there is no reason that a farmer can suspect that the animals is infected (Abernethy et al., 2011; Sarmatino & Enright, 1993).

The study agreed fully with the discoveries thus made; the giving of education to the farm owner helps them to strategize the control measures from knowing the critical times in the epidemiology of the disease. The C-branding of positive animals has the importance of discouraging the farmer from keeping the positive animals, a control at the abattoir for appropriate control measures and the warning to the prospective buyers and auctioneers to discourage from buying or selling the animal. The training also helps to make informed decisions on vaccinations, economics associated with positive animals, what to do in the case of abortions, stillbirths and birth of weak calves. The knowledge also helps them protect their workers, thus improving the ability of the farmer to plan and make precautionary measures.

The results from this study agreed that the disease being a problem increases with the presence of females and to be specific, older females. The control strategies should be concentrated around heifers before gestation. Vaccinations at 6-8 months should be performed, as it is a policy requirement before the animals becomes cows and the disease become difficult to diagnose and control. Therefore, bovine brucellosis control measures should be concentrated on prevention of introduction of the disease, early diagnosis and control. Elimination of disease from older cows should be done through culling of positive reactors before they become chronic. Generally, it is advisable to keep younger cows to prevent disease chronicity in the herd.

The study did not find any significant association between the presence of bovine brucellosis in the cattle herd and symptoms in people. Mangen et al., 2002 argued that the reduction or elimination of bovine brucellosis in livestock is likely to be followed by the reduction in human suffering due to the infection. The reason for this absence of association may be because these symptoms in people are dismissed as old age or relating to something else other than the brucellosis disease. This is associated with the medical staff failure in seeing an association between ailments in people and the diseases of cattle. If the association is not made from the 'farm-animal-relationship' to people, the Human medical staff cannot guess the association and hence these treatment failures. The difficulty of the diagnosis of brucellosis in humans as having been mentioned in literature (Smits, 2013; McDermott & Arimi, 2002) does not encourage routine diagnostic relevance put on the disease by human medical

professionals. 'One Health' interactions should therefore be encouraged as a platform to inform each other about disease developments, especially around emerging diseases to make the team achieve the same goal, that is to prevent human infection and suffering. The State Veterinary services should be used proactively and productively by the human health official's as early disease warning for zoonotic diseases through proactively participations between the two sectors. Proactive actions involve, amongst other things, proactive surveillance for zoonotic diseases, in this case human brucellosis, in cases where the animal sector has indicated a problem.

Furthermore, the animal health professionals (Veterinarians and Animal Health Technicians) should be medically assessed for baseline titres for contagious animal disease, like brucellosis, so that from the employer's side it can be known the stage when the disease was contracted (before employment or in the delivery of services).

The conclusions of the study were: increase in herd size, the number and the proportion of calves to heifers and proximity of the farm to wild life are the main risk factors and the recommendations as discussed above.

The study has proved valuable in identifying risk factors and providing a mechanism for informing control scheme managers of key drivers of infection. It has also highlighted potential weaknesses in the system e.g. lack of branding of test-positive cattle and pointed to the value and need of education for livestock owners. However, as an observational study, it faced the usual limitations of recall bias and the low number of case herds undermined the power of the study.

## **Conflict of interest**

There is no conflict of interest. The study was funded by AgriSETA.

## **Ethical considerations**

All the results from the questionnaire, laboratory results thus accessed, and details of owners will be kept confidential. The findings from this study will be conveyed to the study participants, livestock association during community meetings, 'one health' for human health.

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# Animal ethics approval certificate



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

**ANIMAL ETHICS COMMITTEE**

Private Bag X04  
0110 Onderstepoort

Tel +27 12 529 8434 / Fax +27 12 529 8300  
e-mail: [aec@up.ac.za](mailto:aec@up.ac.za)

**V074-16**

Prof D Abernethy  
Dean  
Faculty of Veterinary Science

Dear Prof Abernethy

**V074-16 : Case control study on risk factors for Bovine Brucellosis in KZN (Dr Nogwebela)**

Thank you for the AEC application.

Since this study does not involve animals, no animal ethics approval is required.

Kind regards

A handwritten signature in black ink, appearing to read 'Elmarie Mostert', enclosed in a simple oval outline.

**Elmarie Mostert**

**Coordinator : Animal Ethics Committee**

Cc Dr N Nogwebela (Researcher)

Prof Matjila (HoD)