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Article

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Abstract: Abattoir workers are liable to zoonotic infections from animals and animal products, primarily to diseases with asymptomatic and chronic clinical manifestations in animals such as brucellosis. No published reports exist on the seroprevalence of brucellosis in abattoir workers in South Africa. Therefore, this cross-sectional study was conducted to estimate the occurrence and risk factors for *Brucella* exposure in abattoir workers in Gauteng Province. A total of 103 abattoir workers and managers from six abattoirs, where brucellosis-positive slaughtered cattle and sheep were previously detected, were interviewed, and tested with serological assays using the Rose Bengal test (RBT), BrucellaCapt, and IgG-ELISA. A pre-tested questionnaire was administered to consenting respondents to obtain information on risk factors for brucellosis. Of the 103 respondents tested, the distribution of female and male workers was 16 (15.5%) and 87 (84.5%), respectively. The seroprevalence for exposure to brucellosis was 21/103 (20.4%, 95%CI: 13.1-29.5) using a combination of RBT, BrucellaCapt, or IgG-ELISA. For test-specific results, seroprevalences by RBT, BrucellaCapt, and IgG-ELISA were 13/103 (12.6%, 95%CI: 6.9-20.6), 9/103 (8.74%, 95%CI: 4.1-15.9) and 18/103 (17.5%, 95%CI: 10.7-26.2), respectively. Low-throughput abattoirs were identified as associated risk as 29.3% of workers were seropositive compared to 12.7% of workers in high-throughput abattoirs, which highlights that direct contact at abattoirs poses higher risk to workers than indirect and direct contact outside abattoirs. This study confirmed the occurrence of *Brucella* spp. antibodies among abattoir workers in South Africa, possibly due to occupational exposure to *Brucella* spp. and highlight the occupational hazard to workers. Furthermore, findings underscore that abattoir facilities can serve as points for active and passive surveillance for indicators of diseases of public health importance. We recommend periodic implementation of brucellosis testing of abattoir workers country-wide to establish baseline data for informing appropriate preventive practices and reduce the potential burden of infection rate among these high-risk workers.

Keywords: Brucellosis; human; serology; abattoir; South Africa

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1. Introduction

It has been established that of all the 1,415 known human infectious pathogens, 61% are zoonotic, and 75% of emerging or re-emerging diseases are also thought to be zoonotic (1). Similarly, 35% and 61% of emerging zoonotic pathogens are known to be transmitted through direct and indirect contact, respectively (1). Apart from abattoirs being facilities where apparently healthy animals are slaughtered to provide wholesome meat for human consumption, abattoirs can also be used to monitor the effectiveness of disease control programmes and disease surveillance (2, 3, 4). However zoonotic diseases pose an occupational hazard to abattoir workers as they will have contact with subclinically-infected animals, in which signs are either absent or too mild to escape diagnoses. Such animals and their products pose a higher risk to abattoir workers as they will not be aware of the pathogens with a greater chance of becoming infected.

Brucellosis is one of the neglected zoonotic diseases and it is endemic in most developing countries with clinical manifestations ranging from asymptomatic (subclinical) to chronic infections in animals (5). It is an important zoonosis that infects humans, and about half a million worldwide are believed to be infected annually (6, 7, 8). Brucellosis causes an immense disease burden on infected individuals in terms of high socioeconomic and financial impact (5, 9). This impact is compounded by the fact that the clinical duration of human brucellosis varies from months to years and the prolonged episodes of clinical illness cause additional losses of time and money owing to disability (10, 11, 12). The risk of brucellosis as an occupational hazard is relatively high among workers in abattoir facilities, meat packaging industries and farm workers (13).

Brucellosis is endemic in the Mediterranean basin, the Caribbean, Africa, south and Central America, Asia, and the Middle East (14, 15, 16, 17). In these areas, *Brucella*-infected animals and their products slaughtered at abattoir facilities can serve as a source of infection to abattoir workers. Workers may be exposed to infection through direct contact with infected animals' secretions or blood as well as aerosol dispersion of *Brucella*-containing droplets through inhalation or contact with the conjunctiva, mucous membranes, or compromised skin especially for individuals without personal protective equipment (PPE) (15, 16, 18). Brucellosis is a complex disease humans can also become infected indirectly by consuming raw or undercooked meat, raw and unpasteurized milk, or milk products (16). A wide variety of risk factors that predispose abattoir workers to infection were identified in studies and include length of days at work; unknown health status of slaughtered animals; non-use of PPE; splash of animal secretion on the face; hand cut injuries during work; lack of hygienic measure in the work environmental; and poor knowledge and attitude of the workers and their perception of zoonoses as occupational hazards (16, 19, 20, 21, 22, 23, 24, 25, 26). These studies were conducted in various countries/regions, each with its unique zoonotic disease profile in abattoir facilities that determine the risk factors identified in each study. In South Africa the only published record focusing on occupational workers exposed to brucellosis is the seroprevalence amongst farm workers exposed to brucellosis case farms that are higher, ranging from 4.0% (BrucellaCapt®) to 16.7% (IgG ELISA®), compared to those exposed to brucellosis negative control farms where seroprevalence ranged from 1.9% (BrucellaCapt®) to 5.7% (IgG ELISA®)(27). There are no empirical data or published reports on brucellosis seroprevalence and risk factors in abattoir workers in South Africa.

Smits, Abdoel (28) indicated that immunoglobulin M (IgM) antibodies against human brucellosis develop early in the infection and remain for several weeks or months. Later, IgG antibodies develop and may be present at lower levels for months to years after the patient recovers. IgG but not IgM antibodies are present in recurring infections (28). Thus, various serological tests are used to detect human brucellosis. Díaz, Casanova (29) encouraged the use of the Rose Bengal test (RBT) as it is a rapid, affordable test adaptable to serum dilutions, which detects IgM, IgG, and IgA in short/acute and long/chronic brucellosis cases. RBT is highly specific in humans with no contact with *Brucella* and is comparable with the BrucellaCapt test to detect the IgM, IgG and IgA. BrucellaCapt is an immunocapture assay recommended to detect relapse of brucellosis in chronic/long cases (30). The IgG ELISA is extremely sensitive serological test for detecting IgG antibodies found in long/acute cases [28]. This study was therefore conducted to obtain evidence-

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based data on the seroprevalence using RBT, BrucellaCapt and IgG ELISA serological tests and associated risk factors for brucellosis in abattoir workers in Gauteng Province, South Africa.

2. Materials and Methods

Study area

The study area is Gauteng Province of South Africa. This is the smallest of the country's nine provinces, accounting for 1.5% of the country's land area and houses about 23.7% of the country's population. The South African human population is estimated to be 58 million with 15 million in Gauteng Province. The country stretches from 22°S to 35°S and from 17°E to 33°E, with a surface area of 1 219 602 km^2 (31). The country has several distinct ecosystems, and it is bounded by 2 798 km of coastline stretching along the South Atlantic and the Indian Oceans. In the north and north-west, its neighbouring countries are Namibia, Botswana, and Zimbabwe and to the east, it is bordered by Mozambique and Swaziland. The mean annual rainfall in the country is 464 mm and the country's topography vary between 1 087 meters to 3 300 meters above sea level, while Gauteng province is about 1 500 meters above sea level. Gauteng Province is divided into three metropolitan municipalities and two district municipalities. The estimated number of livestock in Gauteng Province as of May 2018 was 246 395 cattle, 92 160 sheep, 29 017 goats and 156 264 pigs (32).

Study design

A cross-sectional study was conducted to estimate the seroprevalence of *Brucella* spp. exposure using three serological tests (RBT, BrucellaCapt, *Brucella* ELISA IgG) among abattoir workers in selected abattoirs in Gauteng Province from 2017 to 2018. Six of the 14 abattoirs from a previous study, where slaughtered livestock (cattle and sheep) were confirmed positive for brucellosis (*B. abortus* and *B. melitensis*) (33), were included in the current study. Three of these six abattoirs were high throughput facilities while the other three were low throughput facilities, and all processed multiple species of animals.

Sampling method and recruitment

The study followed a willing participant approach. A total of 103 abattoir workers, managers, and meat inspectors consented to participate in the study. All abattoir workers/managers who met the eligibility criteria (those who were present and consented on the day of sampling) were interviewed. Respondents were classified by the type of duties they performed at the facilities including butcher ('slaughterman'), inspector, transporter, and others (those engaged in any other type of work conducted at the abattoir). A questionnaire that elicited demographic data and duties performed at the abattoir was administered to each participant, when necessary, using interpreters who were members of the research team.

Study population

The inclusion criteria for the study population were being active in the meat industry and working in the abattoir industry. These included the managers and abattoir workers who submitted signed written consent forms. All abattoir managers who signed the consent forms as stakeholders permitted the team to conduct the study at their facilities.

Sample size of abattoir workers

The sample size for human workers in the abattoirs was determined according to Naing, Winn (34) : $n_o = t^2 (p) (1-p)/ d^2$ where t=1.96, p=prevalence, d=precision at a type 1 error of 0.05, n_o=estimated sample size. Since there are no current documented data on the seroprevalence of human brucellosis in abattoir workers in Gauteng Province or South Africa, the prevalence of 50% (0.5) was assumed. For the study, the minimum sample size was determined using the following criteria: p=0.5 and a precision of 10%. For this study, a minimum sample size of 96 was adopted and

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a total of 103 humans was sampled based on seven other workers volunteering to participate and who were available on the allocated day for testing.

Pre-testing of the questionnaire and data collection

A structured questionnaire was constructed with three parts on Microsoft Word Office 2007. This was tested for clarity among five abattoir workers, and content was adjusted based on the responses obtained. The first part of the questionnaire extracted demographic data on the worker comprising the age, sex, number of years at work, job description, and the section where the interviewee was stationed at the slaughter facility. The second part consisted of close-ended questions about attitudinal risky practices during work, such as the use of protective clothing, gloves, eye goggles, masks, eating raw meat, and splash exposure of blood or fluid in the eyes or mouth during work. The last part consisted of questions regarding the subject's perception of the probability of contracting zoonoses at the abattoir and their attitude towards seeking medical assistance whenever they felt any symptoms of illness.

Data collection

All consenting abattoir workers who met the eligibility criteria were interviewed using the structured questionnaire.

Collection of blood samples

A qualified phlebotomist was recruited to collect a maximum of 5 ml of venous blood from each consenting participant following the established procedure during and post-collection of blood samples. A total of 103 blood samples were collected in vacutainer tubes without any anticoagulant to harvest sera and stored under 4ºC prior to usage for the serological assays.

Serological tests conducted

All the serological tests were conducted in parallel and series at the Special Bacterial Pathogens Reference Laboratory of the National Institute for Communicable Diseases (NICD), South Africa.

Rose Bengal test (RBT): The commercial IDEXX *Brucella* antigen (Switzerland) stained with Rose Bengal (30 µl) was mixed with an equal volume of serum sample and the mixture was agitated gently for four minutes at room temperature on a shaker. Standard positive and negative controls supplied with the kit, were treated according to the manufacturer's protocol. Agglutination was read after four minutes, and any visible agglutination was regarded as positive for *Brucella* antibodies. The diagnostic sensitivity and specificity for the RBT used was assumed to be 100% and 75%, respectively, based on previous validation studies (35).

BRUCELLACAPT® (Vircell S. L., Spain): Using the manufacturer's instructions, this single-step immunocapture assay that detects nonagglutinating IgG and IgA as well as agglutinating antibodies (36) was performed, inclusive of the standard positive and negative controls. The reference of sensitivity and specificity of the BrucellaCapt is stated as 80.4% and 96.8%, respectively (36).

***BRUCELLA* ELISA IgG (Vircell S. L., Spain)**: This enzyme-linked immunosorbent assay (ELISA) was performed according to the manufacturer's instructions. Human sera were added to microtitre plates with antigen adsorbed on the polystyrene surface. Unbound immunoglobulins were washed off as the enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After another wash step, the bound conjugate was developed with the aid of a substrate solution (TMB) to render a blue-coloured soluble product which turns yellow after adding acid-stopping solution. The ELISA washer (ELx50/8), ELISA reader (ELx800), and the software program used to read and interpret the results (GEN 5 Software package) were all BioTek (USA) products. The positive and negative controls were supplied with the kit. The reference of sensitivity and specificity of the IgG-ELISA was 75.4% and 100%, respectively (36).

Statistical analysis

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Data were managed using Microsoft Excel sheet v 2016 (Microsoft Corporation, Redmond, USA). All data were entered into the software, and checked by two individuals independently, to check for consistency and errors in data entry. Each of the 17 variables was assessed by univariate analysis for association with *Brucella* positivity status (serology) using the chi-squared or Fisher's exact test in two-by-two tables. The 17 variables were also assessed for significant associations using Pearson's chi-squared test. If two variables were significantly associated, only one more biologically plausible variable was selected for multivariable analysis. Other variables (n=3) were excluded from multivariable analysis due to few participants in one or more categories. Subsequently, only seven of the 17 initial variables from univariate analysis (regardless of p-value) were analysed in Binomial Multivariable Generalised Linear Models following a backward elimination approach. The goodness-of-fit of the multivariable models was performed and compared using the Hosmer-Lemeshow and Omnibus fit tests. Statistical analyses were conducted at 5% level of significance using the 'MASS' and 'rms' packages in R statistical software version 4.2.1 (37).

Ethical approval

Research (V089-16) and Human Ethics approval (519/2017) were obtained from the University of Pretoria Faculty of Veterinary Sciences and Faculty of Health Sciences Research Ethics Committees.

3. Results

3.1. Description of participants

Of the 103 respondents evaluated, the distribution of females was 16/103 (15.5%) and 87/103 (84.5%) for males. The mean age of the respondents was 36.4 years (range 18 - 60). The mean age for females was 32.6 years (range 23 - 49) while for males it was 37.1 years (range 18-60). The distribution of respondents according to age was as follows: for 18 to 30-year-olds, it was 32/103 (31.1%) and for the 31 to 60-year-olds, it was 71/103 (68.9%).

Based on the abattoir-related job specifications, the professionals interviewed were: slaughtermen (butchers) = 68/103 (66.0%), inspectors = 14/103 (14.0%); cleaners = 8/103 (8.0%); offal washers = 7/103 (7.0%); managers = 3/103 (3.0%); transporters = 2/103 (2.0%) and hides and skin processors = 1/103 (1.0%). The numbers of years worked at the abattoir were as follows: work duration of 1 year, 21/103 (20.3%); work duration of 2 years 17/103 (16.5%); and work duration of 3 years and above, 65/103 (63.1%). In addition, according to the number of days at work weekly, 98/103 (95.2%4) worked for 5 to 7 days per week, 2/103 (1.94%) worked for 3 to 4 days, and 3/103 (2.91%) worked for 1 to 2 days.

Analysis of seropositivity by different tests among the abattoir workers

Of the 103 abattoir workers' sera tested, those that were positive on at least one of the serological tests, the seroprevalence for *Brucella* spp. exposure was 21/103 (20%, 95%CI: 13.1 - 29.5). Concurrent test results are as follows: with RBT the number (percentage) seropositive was 13 (12.6%, 95%CI: 6.89 - 20.6); with BrucellaCapt, this was 9 (8.74%, 95%CI: 4.07-15.9) and with IgG-ELISA assay, this was 18 (17.5%, 95%CI: 10.7 - 26.2) (Table 1).

In series, the number (percentage) seropositive with RBT and BrucellaCapt was 7 (6.80%, 95%CI: 2.78 - 13.5), for RBT and IgG-ELISA, this was 10 (9.71%, 95%CI: 4.75 - 17.1), for BrucellaCapt and IgG-ELISA, this was 9 (8.74%, 95%CI: 4.07 – 15.9) and for RBT, BrucellaCapt and IgG-ELISA, this was 7 (6.80%, 95%CI: 2.78 - 13.5) (Table 1).

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Table 1. Overlap and non-overlap of brucellosis serological results for the Rose Bengal test (RBT), BrucellaCapt, and IgG-ELISA testing of Gauteng abattoir workers.

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

*= %-percentage, CI-confidence Interval.

Descriptive statistics and univariable analysis of factors among the respondents predictive of Brucella seropositivity

A total of 17 variables were subjected to univariate analysis for association with *Brucella* seropositivity among abattoir workers (Table 2). The seropositivity on at least one test (RBT, and/or BrucellaCapt, and/or IgG ELISA) among female and male workers during the study period was 6.25% (1/16) and 22.9% (20/87), respectively, and was not statistically significant (p=0.18) (Table 2). Among the age categories, seropositivity was higher (22.5%, 16/71) among older persons of 31 to 60 of age than younger workers of 18 to 30 years of age (15.6%, 5/32), although the difference was not statistically significant (p=0.42) (Table 2). The seropositivity among abattoir workers, stratified by the type of duties performed was as follows: slaughtermen/offal washers (butchers/offal) 22.7% (17/75), and other (inspectors, cleaners, transporters, managers and hides and skin processors) was 14.3% (4/28) and this was not statistically significant (p=0.42) (Table 2). Assessment of other questionnaire responses showed that 8.3% of those who ate raw or undercooked meat were seropositive, while 23.7% of those without knowledge on brucellosis were seropositive to brucellosis; and these were not statistically significant as compared with their corresponding categories (Table 2). According to the type of abattoirs sampled, 29.3% of workers in low throughput abattoirs were seropositive compared to 12.7% of workers in high throughput, this was statistically significant (p=0.03) (Table 2).

Table 2. Descriptive statistics and univariate analyses for factors associated with *Brucella* seropositivity among abattoir workers in Gauteng abattoirs, South Africa.

Variable	Category	Number of humans sampled	Number positive	Percentage of seropositive	Odds ratio (95% CI*)	P-value†
Abattoir type	High throughput	55	7	12.7	2.79 (0.94,9.11)	0.039
	Low throughput	48	14	29.2		
Gender	Female	16	1	6.3	4.44(0.61,197.62)	0.183
	Male	87	20	23.0		
Age category	18 – 30 years	32	5	15.6	1.56 (0.48,6.05)	0.421
	31 – 60 years	71	16	22.5		
Marital status	Married	42	8	19.0	1.15 (0.39,3.57)	0.779
	Single	61	13	21.3		

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Job description	Others	28	4	14.3	1.75 (0.49,7.89)	0.420
	Slaughterer/offal handling	75	17	22.7		
Days at work	1 to 4 days	4	2	0.5	0.24 (0.017,3.53)	0.184
	5 to 7 days	99	19	19.2		
Years at work	More than 1 year	82	16	19.5	1.29(0.32,4.44)	0.663
	Up to 1 year	21	5	23.8		
Working on farms besides abattoirs?	No	65	16	24.6	0.47 (0.12,1.51)	0.164
	Yes	38	5	13.2		
Do you consume unpasteurized milk?	No	77	16	20.8	0.91 (0.23,3.03)	0.865
	Yes	26	5	19.2		
Do you slaughter animals at home?	No	54	8	14.8	2.06 (0.70,6.41)	0.141
	Yes	49	13	26.5		
Do you eat raw or undercooked meat?	No	91	20	22.0	0.33 (0.007,2.49)	0.451
	Yes	12	1	8.3		
Do you always wear your PPE?	No	6	0	0.0	Inf	0.342
	Yes	97	21	21.6		
Do you know of brucellosis?	No	76	18	23.7	0.41(0.07,1.59)	0.265
	Yes	27	3	11.1		
Do you think you can get brucellosis from animals?	No	81	10	12.3	0.33 (0.03,1.57)	0.231
	Yes	22	0	0		
Have you had cut hand-cut injuries on duty?	No	26	6	23.1	0.81 (0.25,2.89)	0.694
	Yes	77	15	19.5		
Have you ever been diagnosed with brucellosis?	No	101	20	19.8	3.98(0.05,320.83)	0.368
	Yes	2	1	50		
Have you ever had animal blood splashed on your face?	No	23	3	13.0	1.92 (0.48,11.25)	0.393
	Yes	80	18	22.5		

*CI-confidence Interval.

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All the 17 variables from univariate analyses were screened for inclusion in multivariable models. The following variables were excluded from the multivariable analysis due to few participants in one or more categories: days at work (98/103 participants worked for more than 1 day); negative previous brucellosis diagnosis (101/103 participants tested negative); regular PPE utilization (97/103 participants used PPE). Other variables were excluded from multivariable analysis because they were significantly associated with at least one other variable: marital status, slaughter at home, job category, work on the farm, consumption of raw meat, blood splash and ever been diagnosed with brucellosis.

Therefore, of the initial 17 variables in Table 2, seven (abattoir, gender, age, drinking of raw milk, years at work, knowledge about brucellosis and hand injury) were included in multivariable generalised linear models and after a backward elimination procedure, data fitted well the model that had only two variables, abattoir and gender (Table 3) (Hosmer-Lemeshow test: $\chi^2 = 0.018$, df=8, p=1.0; Omnibus test: Z=0.59, p=0.539).

Table 3. Multivariable analysis.

Multivariable analysis			
Variable	Category	Odds ratio (95% CI*)	p-value
Abattoir	High throughput (ref)		
	Low throughput	1.19 (1.02, 1.39)	0.027
Gender	Female (ref)		
	Male	1.21 (0.98, 1.49)	0.085

*CI-confidence Interval.

4. Discussion

The research highlights the occupational hazard of diseases such as brucellosis with subclinical infections. Gauteng Province abattoir workers had moderate to low brucellosis seroprevalence, with 20% overall using a combination of serological tests (RBT, BrucellaCapt, IgG ELISA) or 6.8-9.7% using series test combinations (Table 1). The current study was a continuation in abattoir workers after a previous report showed 5.5% brucellosis sero- (using RBT and iELISA) and culture-prevalences (*B. abortus* biovar 1, *B. melitensis* bv 2 and 3 from cattle tissues) in 200 bovines slaughtered in Gauteng Province. Most of the *Brucella* spp. isolated were from animals slaughtered at low-throughput abattoirs (33). The associated risk to workers to brucellosis will be influenced by the brucellosis infection in livestock with which the workers have had contact. This is reflected by the low to moderate brucellosis seroprevalence (4.0% (BrucellaCapt) to 16.7% (IgG ELISA)) of farm workers on brucellosis infected farms compared to low seroprevalence [1.9% (BrucellaCapt®) to 5.7% (IgG ELISA®)] on farms free of brucellosis in South Africa (27). In South Africa, we have limited data on the brucellosis prevalence in animals. A retrospective study reported brucellosis seropositivity using RBT and CFT that ranged from 1.8% to 17.6% in different provinces (3.9% in Gauteng Province) with an overall positivity of 5.8% in livestock from 2007-2015 in South Africa (38). The retrospective study reported brucellosis in livestock assessed at Onderstepoort Veterinary Research laboratory as part of the bovine brucellosis scheme in South Africa. The bovine brucellosis scheme focuses mainly on high-risk bovine and is voluntary for other bovine and livestock (39) and thus, does not reflect brucellosis seropositivity at abattoirs since these do not require bovine brucellosis status for slaughtering. Both these studies (33, 38) report brucellosis prevalence in a small percentage of livestock in the country as is the case of humans (n=103) tested in the current study. These studies provide a limited view of the prevalence of brucellosis in both livestock and humans in South Africa, which needs to be expanded. However, these studies reflect the endemic nature of brucellosis in livestock in South Africa which poses a higher risk to humans in contact with infected animals. In addition to the human brucellosis seroprevalence reported in the current study, the associated risk factors such as gender and abattoir type highlight that direct contact with animals and their products

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are important risk factors for abattoir workers rather than direct and indirect contact with animal products outside the abattoir facilities.

In animals, a presumptive diagnosis is made by assessment of specific cell-mediated or serological responses to *Brucella* antigens (40). Furthermore, no single serological test is suitable for each or all epidemiological situations (40). This in part, requires screened animal samples that are reactive to be tested using established confirmatory or complementary procedures (40). Thus, no single serodiagnosis test can be used to diagnose brucellosis in livestock and a combination of serological tests and/or culture and molecular methods is required (41).

In humans, the clinical presentation of human brucellosis can be ambiguous, as such presumptive identification of infections is conducted based on morphologic, cultural, and serological properties (7). Supportive evidence of human brucellosis diagnosis may include, a demonstration of *Brucella* antigen in human blood using validated serological tests, as well as an indication of high sustained IgG antibody titre as seen in agglutination, complement fixation test or ELISA with standardized antigen (16). However, there are no routine brucella antigen detection tests in humans in South African. There is also no consensus on the evaluation of preferred single serodiagnosis tests for human brucellosis, and this is in part made difficult because there is no single unquestionable test for defining the disease against which all other laboratory assays should be measured (42). However, other diagnostic methods that can be used are culture and PCR to detect pathogen nucleic acid. Serological tests for brucellosis are frequently evaluated by comparing results with those obtained with other serological assays, used alone or in combination (42). As mentioned, this in part is a result of the evolution of the disease either in the acute or chronic phase. IgM is present in acute cases and this immunoglobulin reverts to the background levels and the IgG (IgA) dominates later in the chronic phase of the disease (29). The immunoresponse to brucellae is dominated by antibodies to the polysaccharide (PS) section of smooth (S) *Brucella* lipopolysaccharide (S-LPS), and this elicits IgM/IgG (IgA) shift (43). Multiple tests for confirmation are also used to eliminate cross-reactivity of serological tests to other Gram-negative bacteria such as *E. coli* O:157, *Francisella tularensis* and *Yersinia enterocolitica* 0:9 (44). All this had to be considered based on the three serological tests used in this study to detect smooth *Brucella* antibodies in abattoir workers. For a reliable serological diagnosis of human brucellosis, at least two tests are required: one based on a high-sensitivity screening method, and another based on more specific strategies to confirm the preliminary test results.

The RBT is a rapid and sensitive screening test that determined 12.6% seropositivity in abattoir workers from this study. RBT can produce false positive results, especially in patients with cross-reactive organisms and healthy individuals who may have had contact with S-*Brucella* without developing the disease (16). The screening RBT is recommended to be confirmed with other serological tests. In this study, RBT confirmed with IgG ELISA reported 9.7% seropositivity compared to 6.8% of RBT and BrucellaCapt (Table 1). We reported a lower seropositivity of 6.8% with RBT confirmed with both BrucellaCapt and IgG ELISA in series (Table 1). IgG ELISA (17.5%) as a parallel test recorded higher seropositivity compared to both BrucellaCapt (8.7%) and RBT (12.6%) (Table 1). Further, the seropositivity (17.7%) recorded with parallel IgG ELISA was lowered (8.7%) when combined in series with BrucellaCapt. The evolution of immunoglobulin isotypes can be measured following infection and treatment using ELISA (16).

Xu et al (2023) (45) evaluated BrucellaCapt and IgG ELISA in patients with suspect brucellosis and found that BrucellaCapt had excellent specificity (100%) and high positive predictive value (PPV) (100%), with sensitivity and negative predictive values of 88.3% and 86.3% and found the combination of BrucellaCapt and IgG ELISA had excellent diagnostic prediction. The current study recorded that IgG ELISA plus BrucellaCapt both had 8.7% seropositivity which supports the evaluation of human brucellosis in an endemic area by six serological tests (RBT, standard serum agglutination (SAT), Brucella Coombs, BrucellaCapt, IgM ELISA and IgG ELISA) that found all tests valuable for positive results, but only Brucella Coombs and BrucellaCapt were reliable for negative serological results (46). Another study tested human brucellosis in an endemic area in Turkey (46) and reported that BrucellaCapt recorded higher seropositivity (74.0%) at 1/360 titre cut-off and

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80.9% at cut-off titre value at 1/160 compared to other serological tests (46). For reliable serological human brucellosis diagnosis, as is the case with serological animal brucellosis, at least two tests are needed consisting of a highly sensitive screening test followed by more specific methods to confirm the preliminary screening results. From our results the combination of Ig ELISA and BrucellaCapt approach might be the most accurate reflection with no false positive (Table 1).

Two abattoir workers indicated a previous diagnosis of the disease and one tested positive with RBT and BrucellaCapt, while the other tested negative with all tests. A study reported that the serological cure rate increased from 8.3% in the first three months to 71.4% after 2 years (47) as the IgM becomes non-detectable and low levels of IgG occur. The median time of serological cure was 18.5 months and 28.6% of cured patients continue to have high titres two years or more. Thus, the positive individual is plausible because brucellosis requires prolonged treatment, and the antibodies remain in the system for a very long time, even after treatment (16).

This work is the first abattoir worker-based study on human brucellosis to be conducted in South Africa, thereby providing relevant baseline data on this group. These findings also underscore the urgent need to replicate the study in other provinces in South Africa, to fully understand the exposure status among the occupationally exposed groups in the country. This is imperative because brucellosis is endemic in livestock in the country (33, 48, 49, 50), and *B. melitensis* has been isolated and reported in humans (51).

The seroprevalences of 17.5% (ELISA) and 12.6% (RBT) in this study were comparable to a range of seroprevalence reports from other studies around the world, of which seroprevalence of 21.7% (ELISA), 25.5% (ELISA) and 19.5% (RBT) was observed among abattoir workers in Pakistan, India and Tanzania, respectively (19, 52, 53). Although, these are comparatively higher than the seroprevalence obtained by the use of ELISA and RBT in our study, some other studies have in contrast reported lower seroprevalences of 7.9% (ELISA) and 9.4% (RBT) among abattoir workers in Iran and Egypt, respectively (20, 54). However human brucellosis seroprevalence in abattoir workers will vary depending on the prevalence of the disease in livestock (54, 55), infrastructure, training, sanitation and/or hygiene standards at abattoirs. This includes awareness of the disease and training on the use of PPE (20, 21, 22, 25, 54, 55) job description (slaughterers or butchers and workers with cut injuries have higher risks (16, 53)) and consumption of unpasteurized milk products and raw meat (22, 23, 24, 56). Risk factors differed from study to study indicating that these factors influence the disease prevalence in humans differently in each area.

The risk factors associated with abattoir workers identified in this study, namely, gender and abattoir type, which only reflect direct contact with animals and products were unexpected. Brucellosis is a complex disease and we thought that direct contact with animals and especially indirect contact with animal products such as drinking raw milk, eating raw meat and slaughtering at home as reported in the literature (21, 22, 43, 57, 58) would have been an associated risk factors for these workers. There was no statistical significance in risk factor among workers that that drank raw milk (19.0%), consumed raw or undercooked meat (22.0%) and those who slaughter animals at home (25.5%) in comparison to those who did not engage in these acts (Table 2). Informal markets in developing countries could create a bias as it might be perceived by interviewees that purchasing milk and meat issues might pertain to quality standards, for example as regulated by the Agricultural Product Standard Act of 1990 (Act no 119 of 1990) in South Africa. The act regulates that milk must be pasteurized and meat chilled at appropriate temperatures which formal businesses adhere to; however, this is not always the case with informal businesses such as corner groceries, spazas, takeaways, and restaurants. These informal businesses could sell raw milk and meat not properly chilled or before a drop in the pH of the meat, because *Brucella* dies off rapidly when the acidity drops below pH 4, and very rapidly below pH 3.5 (16). This is a limitation in the questionnaire and could be reduced by including a question to determine if milk and meat products are obtained from informal or formal markets.

The participation of both genders in the abattoir activities as observed in our study is of significance. Out of the 103 workers tested, females and males accounted for 15.5% and 84.5%, respectively. A higher seropositivity was recorded in male (22.9%) compared to female (6.25%)

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workers, and the odds of males becoming infected with *Brucella* spp. was about 0.18 times more than the odds of females. The 95.2% (20/21) proportion of seropositive males in our study is comparable to the 87.0% reported for male workers in abattoirs in Nigeria (21). This in part showed bias as there were more males than females working in the abattoirs. The participation of women in the economy of South Africa has been on the increase which has contributed to the empowerment and improvement of women's means of livelihood and enables women to contribute to the economy of the country as a whole (59). Therefore, it cannot be over-emphasized that the increase in female members of the population working in high-risk jobs in the case of abattoir workers, has the potential to increase their exposure to brucellosis and other zoonoses in the future in the country. The marital status of the workers reported that those who were married had 19.0% seropositivity and those in the single category, reported 21.0% seropositivity. This variable of marital status may not have any significance in the transmission of the disease to spouses, because human to human transmission is very uncommon (60).

According to the type of abattoirs sampled in this study, it was recorded that 12.7% and 29.2% of the staff were seropositive (p-value = 0.03) at high-throughput and low-throughput abattoirs, respectively. Higher prevalence in cattle slaughtered in low-throughput abattoirs (22.4%), compared to high-throughput abattoirs (5.2%) was reported in cattle slaughtered in 14 Gauteng Province abattoirs (33). Workers from these abattoirs were selected for the current study. It is an important finding as one would expect that high-throughput abattoirs might have a higher risk as low-throughput abattoirs slaughter a maximum of 20 animals per day while high throughput abattoirs slaughter more than 20 to the maximum set by the capacity of the facility. However other factors, such as better infrastructure, training, sanitation and/or hygiene standards at high-throughput abattoirs could lead to a lower risk, which should be investigated. It cannot be over-emphasized that interventions need to be instituted to reduce the impact of identified risk factors as part of an overall objective to reduce the exposure potential and exposure experience of abattoir workers to brucellosis.

5. Conclusions

This study has determined, for the first time in South Africa, the occurrence of antibodies against *Brucella* spp. among abattoir workers, which could reflect current infection or previous exposure to *Brucella*. The risk factors associated with brucellosis seropositivity indicated that contact with animals and their products at abattoirs poses an occupational risk. We recommend implementing country-wide brucellosis testing of abattoir workers to establish baseline data that could indicate previous exposure, which could be used to mitigate and motivate appropriate preventive practices to reduce the infection rate among these high-risk workers. Finally, such an approach will also provide insight into the magnitude of infections by *Brucella* spp. among abattoir workers. The evidence-based data provided by our study provides baseline data for policymakers in decision-making. The findings of this study underscore that abattoir facilities can be used for active and passive surveillance of some diseases of public health and economic importance.

6. Limitations of the study

Isolation of the infecting *Brucella* spp. could not be conducted. Certain bacterial pathogens (*Yersinia enterocolitica* O:9, *Salmonella enterica* serovar Urbana O:30, *Francisella tularensis*, *Escherichia coli* O116 and O157, *Vibrio cholerae*, *Xanthomonas maltophilia*, and *Afipia clevelandensis*) (50, 51) that can cause false positive serological test results for brucellosis due to cross-reactivity might limit this serological study. Our sample size may also be considered small, but it met the requirement for statistical evaluation. A nationwide analysis, which utilizing a larger sample size, may present a more accurate result. The number of female workers was small compared to male workers, hence could lead to bias.

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JF, LB, HG, were involved in the analysis of the experimental work, manuscript preparation and laboratory tests. All authors read and approved the final manuscript.

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