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SERO-Prevalence and Associated Risk Factors of Brucellosis in Camel Slaughtered at Akaki Abattoir, Central Ethiopia

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Abstract

Background *A serious zoonotic illness, brucellosis has an effect on public health and the economy, especially in poor countries. At the Akaki Abattoir in central Ethiopia, this study looked into the seroprevalence of brucellosis in camels as well as the risk factors that are linked to it.*

Results *The study revealed that out of the total 210 samples tested for RBPT, 6 (2.8%) were found to be positive, of these, 5 (2.4%, at 95 % CI: 0.05-0.51) were found to be seropositive for brucellosis after further CFT testing. Based on both RBPT and CFT, there was a significant difference in the seroprevalence of camel brucellosis between the sexes ($\chi 2=7.99$, $p<0.05$). The seroprevalence was significantly higher in females compared to male animals. Whereas the association between brucella antibody distribution and the corresponding risk factors (age, p= 0.093, Bcs p=0.325) were noticed to be statistically insignificant*

Conclusions *Overall, the seroprevalence of camel brucellosis in the research area was low within the parameters of the current investigation. However, as the study is characterized by limited sample size, the current finding as pioneer research should be considered as indicative on the presence of brucellosis among the camel population. Therefore, future studies having increased focus on female camels, ought to be conducted under coordinated nationwide epidemiological surveillance.*

Keywords*:* Rose Bengal Plate Test (RBPT), Risk Factors, Complement Fixation Test (CFT), Seroprevalence, Camel Brucellosis

1. Introduction

The camel (Camelus dromedarius, one-humped camel) is an essential socioeconomic element of the pastoral and agricultural systems in arid and semi-arid regions of Asia and Africa. [1]. Ethiopia has an estimated 2.3 million camels, which puts it third in the world behind Sudan and Somalia. [2]. Camels in these regions are mainly kept for milk and meat production [3,4]. In the pastoral herd, camels do not perform well, despite their enormous potential for productivity. These locations' camels may be susceptible to brucellosis and other cross-transmitted diseases due to a number of risk factors. [5]. Brucellosis is a highly contagious bacterial zoonotic disease that is significant for worldwide public health. It is sometimes referred to as Undulant Fever, Malta Fever, Contagious Abortion, or Bang's Disease. [6]. It is brought on by members of the Brucella genus. These are Gram-negative, facultative intracellular, non-motile, aerobic coccobacilli. Brucella's capacity to proliferate and endure in host cells is intimately related to its ability to cause chronic disease and elude innate and adaptive immunity. [7]. Ten species of Brucella are currently known, including the more well-known six classical species: Brucella suis (pigs, reindeer, and hares, biovars 1–5), Brucella ovis (sheep), Brucella canis (dogs), Brucella neotomae (desert wood rats), and B. abortus (cattle, biovars 1–6, and 9). Recent discoveries of Brucella microti (voles), Brucella inopinata (reservoir unknown), and Brucella ceti and pinnipedialis (dolphins/porpoises and seals, respectively) have added new species to the genus [8]. Brucella ovis, Brucella melitensis, and Brucella abortus can all cause the disease in dromedary camels [9]. However, Brucella melitensis and Brucella abortus are the sources of brucellosis, which frequently affects them. especially when they are pastured together with infected sheep, goats and cattle [1,10]. The pastoral management system's enormous herd size, sharing of watering spots with ruminants, and poor hygiene procedures encourage the spread of camel

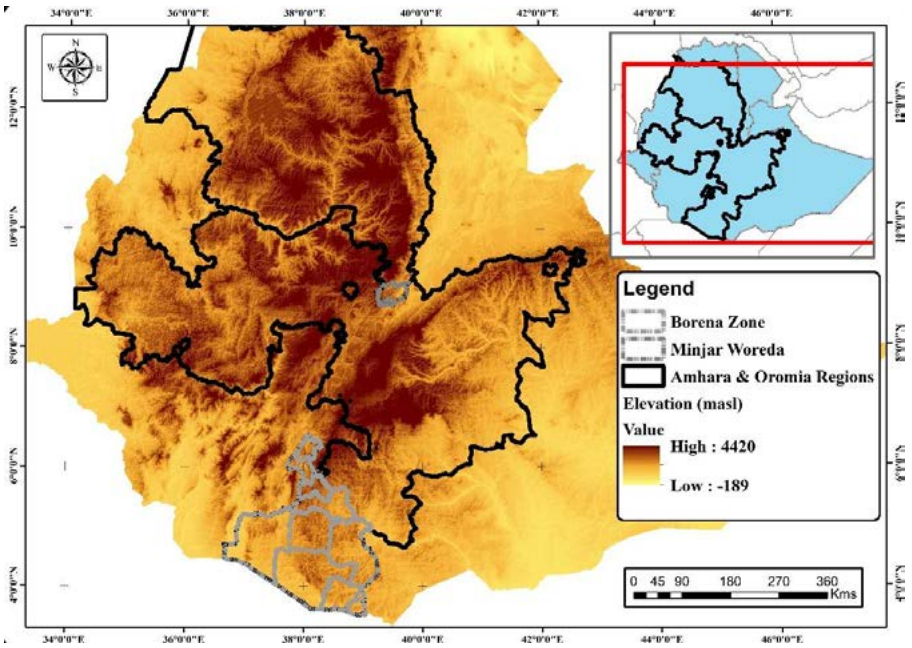
brucellosis, especially during pregnancy or childbirth, by an infected female [10]. Due to poor herd fertility, a long calving interval, a late first calving age, and relatively low milk output, brucellosis can typically result in a significant loss of productivity in camels [11,12].

The illness hinders the free movement of animals and creates a barrier to the import and export of animals, which limits the cattle trade [13]. Furthermore, it should be mentioned that human brucellosis poses a serious risk to public health because it causes undulant fever, an acute febrile illness that can develop into a more chronic form and cause major complications that affect the cardiovascular, musculoskeletal, and central nervous systems [14]. The regions of Sidamo, Harar, and Tigray in Ethiopia reported the first cases of camel brucellosis, with a seroprevalence of 4.4% [15]. Since then, research on the prevalence of brucellosis has been carried out in a few locations around the nation. According to Seroprevalence studies conducted in different camel rearing areas of the country, overall prevalence rates of 4.2%, 5.3%, 4.1%, 3.1%, 2.43% , and 4.5% were reported [5,16-20]. There is a greater knowledge vacuum regarding the disease's prevalence, nevertheless, given the possible damage it poses and the strategic value of camels to Ethiopia's pastoralist society. In addition to being restricted to specific regions, Ethiopia's current research is also extremely dispersed and constrained. Furthermore, the Ethiopian scientific community continues to overlook camels as one of the domestic livestock [21]. Hence, the current study intends to ascertain the apparent prevalence of camel brucellosis and evaluate potential risk variables linked to camel brucellosis seropositivity in camel populations brought from Ethiopia's Borena, Kereyu, and Minijar regions.

2. Materials and Methods

2.1. Study Area

The Akaki Abattoir served as the study's site. This is situated on Addis Ababa's southern outskirts. It is located in Ethiopia's central highlands and has coordinates of 8°52'7"N 38°47'5"E. With a noticeable rainfall peak in July and August and a rainfall minimum throughout the boreal winter (December to February), the city has a mild climate. Summertime sees the lowest average monthly temperatures, which range from 10 to 20 degrees Celsius [22]. Camels from the Ethiopian regions of Borena, Kereyu, and Minijar were brought to the Akaki Abattoir to be slaughtered. About 600 km south of Addis Ababa, in the Oromia National Regional State, sits Borena, the primary source of study animals. The Borena zone has a semiarid climate [23]. Kereyu is roughly 250 kilometres east of Addis Ababa in the Oromia National Regional State. Kereyu has a dry climate in general. Minijar is roughly 130 kilometres east of Addis Ababa in the North Shoa administrative Zone of Amhara National Regional State. Minijar experiences dry weather most of the time (National Meteorology Service Agency (NMSA) [24].



Source: Prepared Based on Data by (UNOCHA, 2024).

2.2. Study Population

The camels that were slaughtered at the Akaki Abattoir were privately owned. They were driven there from their home regions and held in the lairage for a period of one to seven days. The study was conducted during this period. Seven camels were slaughtered on average each day and 2,500 annually in the abattoir. In all, 210 camels (male and female) were used in this investigation. The study animals were categorized in to two age groups; adult (5-15 years old) and old age group (15 > years old). Based on their body condition score they were categorized as camels having good body condition and those having poor body condition.

The camels that were slaughtered at the Akaki Abattoir were driven there from their home regions and held in the lairage for a period of one to seven days.

2.3. Study Design

From December 2017 to April 2018, a cross-sectional study was conducted in Akaki Abattoir to ascertain the seroprevalence of camel brucellosis. Census sampling method was employed for higher degree of accuracy in the data. All camels presented in lairage on each visiting day were sampled. During sample collection, all necessary risk factors related to camel brucellosis (age, sex, and body condition) were properly taken.

2.4. Sample Size Determination

The formula provided for census sampling methods was used to calculate the study animals' sample size [25]. Using 4.5% as the anticipated prevalence [5].

$$n = \frac{1.96^2 P_{exp}(1 - P_{exp})}{d^2}$$

Where,
n = required sample size;
p = expected prevalence (p =4.5% =0.045);
d = 5% = 0.05, 1.96 (CI = 95%). Thus, the desired sample size for p = 0.045 was n = 66. But for precision purpose the sample size was raised to 210.

2.5. Sample Collection and Processing

A sample collection form was created beforehand to record the essential risk indicators for camel brucellosis, including age, sex, and physical condition score. Using simple vaccutainer test tubes, an approximate 8 ml blood sample was extracted from jugular vein punctures in animals that were adequately secured and kept in an ice box. There were no anaesthetics used at the time of the sample collection. The blood samples were shipped in a leak-proof container with ice packs to the Microbiology Laboratory at Addis Ababa University College of Veterinary Medicine. They were then centrifuged for five minutes at 1000 rpm. After that, the sera were transferred into 5-milliliter cynovial tubes and kept in a refrigerator at -20°C until they were processed further.

2.6. Serological Tests

The specificity of the serological tests used for the diagnosis is crucial for the control, eradication, and surveillance of brucellosis; in these situations, the highest specificity tests are needed, or the use of at least two tests administered serially is typically advised for maximal specificity [26]. Cross-reacting bacteria like *Salmonella serotypes, Yersinia entrocolitica,* and *Escherichia coli* have the ability to influence serological results when tests with limited specificity are employed [27]. There is currently no established methodology or titer for diagnosing brucellosis in camels. The test protocol described for the diagnosis of bovine brucellosis should be used for camels, according to [28]. A combination of Rose Bengal Plate Test and Compliment Fixation Test is generally accepted and most widely used serial test schemeFor an animal to be deemed infected in these serial testing techniques, it must respond positively to both tastes [26]. Both testes were done in the National Veterinary Institute. All sera samples were submitted to the institute and were initially screened by RBPT. Sera and antigen were processed according to suggested protocol after being allowed to stand at room temperature for 30 minutes before to the test [29]. Each circle on the plate received around 30 µl of test serum, and an equivalent volume of RBPT antigen (*B. abortus* suspension) was added next to the serum. A wooden applicator was used to completely mix the antigen and test serum, and the mixture was rocked for three to four minutes. Lastly checked for obvious agglutination CFT was used to confirm positive sera with RBPT utilising the Standard *Brucella abortus* antigen. The methods described by and were followed for the proper CFT test and reagent preparation. A serum was deemed CFT positive if it fixed 50% of the complement at a dilution of 1:5 or higher [28,29].

2.7. Data Analysis

The Microsoft Excel spreadsheet program was used to store the row data derived from the serological testing. Software called STATA Version 16.0 was used to calculate descriptive statistics. To determine whether there was a statistically significant correlation between the risk factors and the serological results, Pearson's chi square test was employed. The logistic regression method was used to interpret the degree of relationship between the various putative risk factors and prevalence. A result was deemed statically significant if its 95% CI and P-value were less than 0.05. If the computed $p<0.05$ and the 95% CI for OR does not contain one of its values, there is a statistically significant relationship between the variables.

3. Results

In the current study, out of 210 samples tested for RBPT, 6 (2.8%) were positive reactors. Five (2.4%) of them were found to be seropositive for brucellosis after further CFT testing. The result of Rose Bengal Plate test and Compliment fixation Test on those sera that had positive reaction to both tests was used as true positive and thus indicated 2.4% overall seroprevalence of brucellosis in the tested camels. Based on both RBPT and CFT, the predicted seroprevalence of putative risk variables showed a significant difference in the seroprevalence of camel brucellosis between the sexes ($\chi2= 7.99$, $p<0.05$). The seroprevalence was significantly higher in females (6.2%) as compared to male animals (0.7%) as indicated in Table 1. On the other hand, the association between the presence of *brucella* antibody and the corresponding risk factors (age, p= 0.093, BCS p=0.325) were noticed to be statistically insignificant, but this study result shows that the prevalence in different age group is 2.1% in adult and 2.9% in older animals. Higher seroprevalence was recorded in camels with poor body condition (4.2%) than under the category of good body condition score (1.9%) as indicated in Table 2.

Risk factor	No of tested Animals	No of positive Animals (%)	χ2 Value	P-value
Sex Female	64	4(6.2%)	5.93	0.015*
Male	146	1(0.7%)		
Age Adult	141	3 (2.1%)	0.12	0.731
Old	69	2 (2.9%)		
BCS Poor	48	2 (4.2%)	0.85	0.356
Good	162	3 (1.9%)		

χ2= chi square *= There exist statically significant difference

Table 1: Association of Different Risk Factors with Brucella Seropositive Camels

Risk factor	No of tested Animals	No of positive Animals (%)	Crude OR 95% CI	Adjusted OR 95% CI
Sex Female	64	4(6.2%)	1	
Male	146	1(0.7%)	0.12 (0.01-0.94)	0.11 (0.01-0.92)
Age Adult	141	3 (2.1%)	1	
Old	69	2 (2.9%)	1.37(0.22-8.41)	1.17(0.17-7.81)
BCS Poor	48	2 (4.2%)	1	
Good	162	3 (1.9%)	0.43(0.07-2.67)	0.42(0.06-2.79)

χ2= chi square

Table 2: Multivariable Logestic reggression analysis of brucellosis with potential risk factors

4. Discussion

Compared to other livestock animals in the same regions, camels are relatively resilient and less prone to numerous diseases [30]. However, they may be more likely to experience severe health issues as a result of the extremely hard and stressful circumstances regarding temperature, water availability, and nutrition [31]. Many infectious diseases that plague camels might pose a risk to their handlers because camel herders have a deep cultural bond with their animals, which is demonstrated through intricate practices [32]. Considered one of the most important human health problems in the world, brucellosis is a major zoonotic disease that affects both humans and domestic animals, including camels [33]. Compared to extensive management systems, camel brucellosis is more common in intensive camel production systems, where many animals are housed in close quarters on a farm [10]. Typically, camels from the Borena, Kereyu, and Minijar regions are brought to Akaki Abattior for slaughter. In these regions, camels are raised for traction, milk and meat production, transportation, and a number of other socioeconomic uses [32]. 210 camels that were clinically normal at the time of sampling were sampled for the current study; however, due to the owners' unavailability, it was not possible to review the camels' prior clinical symptoms of brucellosis. According to the current investigation, the overall seroprevalence of camel brucellosis was 2.4%. This finding agrees with the results recorded by in Jigjiga and babile district with a prevalence of 2.43%, in Somali with a prevalence of 2.8%, in Afar, with a prevalence of 2.09%, in Borena with a prevalence of 1.8%, in Dire Dawa with a prevalence of 1.6% [16,17,20,34,35]. However, the current study finding disagreed with earlier report in same study area with a prevalence of 4.2% [5]. also, this study result disagree with who report that the prevalence in Afar is 4.1%, In various areas of the Afar region, and recorded prevalences of 5.7% and 7.6%, respectively [36-38]. It is also in disagreement with prevalence of 23.8% reported by in Darfur, Western Sudan, a prevalence of 2.0 to 15.4% reported by in Kenya, 19.4% reported byin Jordan, 30.5% reported by in Sudan, 7.61% reported by in Egypt [39-43]. Topography and management systems may be the cause of these elevated prevalence rates.

However, the current study's observation is greater than the 0.9% that recorded in the Oromia region's Borena [44]. The size of the herd, sample size, management techniques, the existence or lack of infectious foci, such as herds infected with Brucella, which could spread the disease to contact herds, and the unpredictable environmental conditions of pastoral and agro-pastoral areas, which can lead to stress and disease recurrence, can all affect the seroprevalence of camel brucellosis [5,18]. Use of tests with low diagnostic sensitivity also contributes for the variability of the results. The sensitivity and specificity of the serological assays are significantly impacted by the existence of false positive serological cross reactions brought on by other gram-negative bacteria. RBPT is regarded as a satisfactory screening test [28,45]. The use of CFT as a confirmatory test in serial testing was justified by its high specificity [28]. Consequently, the effectiveness of brucellosis detection

is increased by using a serial testing approach that first screened all samples using RBPT and then applies CFT to positive reactors, as used in the current test [16]. In the current investigation, females had a comparatively greater seroprevalence of brucellosis (6.1%) compared to males (0.7%). Sex-related differences are statistically significant ($p<0.05$), which is consistent with the findings of and [5,46-48]. Erythritol, which promotes the growth of B. abortus, may be the cause of the higher prevalence of brucellosis in female camels. Through their milk and uterine discharge, female animals are also crucial in spreading the disease. Additionally, lactation, pregnancy, and other reproductive stressors are linked to the relaxation of immunity in female camels, which raises the prevalence in females [49]. The prevalence of brucellosis among different age groups found to be 2.1% in adult and 2.9% in older animals. The slight variation in the seroprevalence may be caused by the small number of older camels slaughtered at the abattoir. Although, the result indicates that, the older ones have higher brucella antibody concentration than the younger ones, this agrees with the fact that in essence, brucellosis affects sexually mature animals, and as sexual maturity develops, so does vulnerability since erythritol and sex hormones have an impact on the disease's pathophysiology. Despite the possibility of a few latent illnesses, young animals often have higher infection resistance and clear infections more easily [50]. Immunity to a variety of infectious diseases is greatly influenced by nutrition. It is anticipated that animals who are underfed will have weakened immunity, which will show up as poor physical condition [11,51]. Thus, camels with a low body condition score (4.2%) had higher seropositivity than camels with a high body condition score (1.9%). found similar results in Tanzania. Limitation The camels brought for slaughter to the abattoir were owned by local merchants which are not available, so it makes it very difficult to distinguish the origin of the animals furthermore animals slaughtered at the abattoir were apparently healthy animals so non infected animals might dominate the study population, in this case animals representing the real population with different prevalence might be missed from the study population Therefore, further studies need to be done at the origin level [52,53].

5. Conclusion

One of the most common zoonoses in the world, brucellosis is a highly contagious and economically significant bacterial illness of animals. According to the current study, there is a low overall seroprevalence of brucellosis in camels in the population under investigation. The main risk factor for the disease's development was determined to be sex, but age and body condition score did not significantly correlate with Brucella seropositivity. Despite the low seroprevalence of camel brucellosis, seropositive animals could become future infection foci, endanger public health, and reduce camel productivity and market value. Information about the disease's epidemiology in the nation is lacking, despite the fact that brucellosis is common in camel populations.

Animal ID	Date of collection	Sex of the animal	Age of the animal	Body condition of the animal	Tests	
					RBPT	CFT

Annexes

Annex I : Sample collection sheet for laboratory analysis

Annex II: Test procedures

Rose Bengal Plate Test

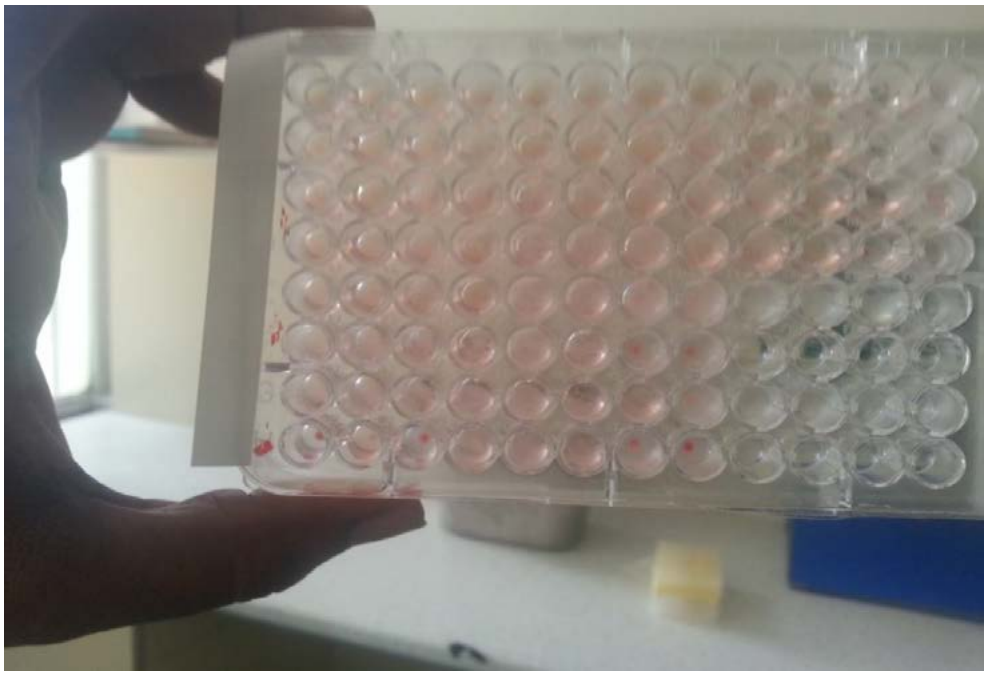
- Each circle on the plate received 30 µl of RBPT antigen, 30 µl of test serum was added next to the antigen, and the two were properly mixed with a wooden applicator. The plate was then shacked for four minutes.
- The degree of agglutination reactions was measured after four minutes and recorded as follows: + + + + (coarse clumping and clearing), + + + (clumping and some clearing), + + (visible fine agglutination), + (weak fine agglutinations using magnifying glass), and in the case of positive reactions, 0 (no agglutinations).
- Source [29]
- Complement Fixation Test (CFT)
- To inactivate the native complement, the sera were prediluted to 1:5 and incubated for 30 minutes at 580°C in a water bath.
- The wells of the first and second rows of the U-bottom plate were filled with 25 µl of diluted test sera, and all wells save the first row were filled with 25 µl of veronal buffer.
- After that, 25 µl of serum from the second row was transferred to create serial doubling dilutions, which continued for at least four dilutions.
- Antigen was diluted to working dilution in 25 µl, with the exception of the anticomplementary controls, who were given 25 µl VCM.
- All wells, with the exception of the control wells, received 25 µl of complement in working dilution.
- Serum control contains serum + complement + diluent, while antigen control has antigen + complement + diluent. Before the haemolytic system was added, the complement control had complemented + diluent, and the haemolytic system had diluent set up to include 75 µl total volume in each case.The plates were incubated at 370C for 30 minutes with agitations (warm fixation)
- Each well received 25 µl of a volume-sensitized 2% SRBC

solution. After sealing, the plates were incubated again for 30 minutes at 370C while being stirred.After the plates were centrifuged for four minutes at 2500 rpm to deposit unlyzed red blood cells, the data were read.

- Interpretation: Sera with a strong reactivity were categorised as positive if they had at least 50% fixation of complement (2+) at a dilution of 1:10 and above, or more than 75% fixation of complement (3+) at a dilution of 1: 5 [28,29].

Annex III: Images displaying the outcomes of the complement fixation and rose Bengal plate tests

Rose Bengal plate test with visible fine agglutination



Complement fixation test with visible fixation of complement

Declarations

Ethics Approval and Consent to Participate

Name of the Ethics Committee

Animal Research Ethical Review Committee, Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia.

Consent to Participate

The majority of the camels slaughtered at the Akaki abattoir in Addis Ababa, Ethiopia, come from pastoral regions such as Kereyu, Borena, and Borana. In particular, the Akaki abattoir slaughters camels and other animals, such as sheep and goats, and provides meat to customers. These camels are owned by pastoralists from the aforementioned areas who take their animals to the slaughter and leave them there. As such they were unavailable at the time of the study and we were unable to receive their consent.

Consent for Publication

Availability of Data and Materials

Due to resource constraints, the datasets created and/or examined during the current work are not publically accessible; however, they can be obtained from the corresponding author upon reasonable request.

Competing Interests

I would like to explicitly state there are no any competing interests with respect to the submitted work.

Funding

No Funding

Authors' Contributions

FT conducted all aspects of the study, including the formulation of the research question, study design, data collection, data analysis, and manuscript writing. He was responsible for all elements of the research process and prepared the manuscript for submission.

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