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Prevalence of Brucellosis among Febrile Patients Attending Kampala International University Teaching Hospital, Southwestern Uganda

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Abstract

Brucellosis is a zoonotic disease mainly acquired through consumption of infected animal products such as milk and meat. It is one of the leading zoonotic diseases and is a serious public health concern in endemic areas. Specifically, the study aimed at determining the prevalence, antibiogram, comorbidities and factors associated with Brucellosis among febrile patients attending Kampala International University Teaching Hospital. A cross sectional study to determine the prevalence, antibiogram and comorbidities of Brucellosis among febrile patients attending Kampala International University Teaching Hospital, South-western Uganda was carried out from May to July, 2023. Blood culture, Modified Kirby-Bauer, well-structured questionnaire and Clinical case notes were methods used. Results from the study indicated that out of the 195 febrile participants sampled, 5(2.6%) tested positive for Brucellosis while the remaining 190(97.4%) tested negative for the disease. Prevalence of Brucellosis was low.

Keywords*: prevalence, brucellosis, febrile patients*

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Introduction

Brucellosis is a zoonosis caused by *Brucella species*.[1-3]With more than 500,000 new cases reported per year, brucellosis is still a major problem for people around the world.[4] *Brucella suis*, *Brucella melitensis*, and *Brucella abortus*, which also cause Brucellosis in goats, cattle, and pigs, respectively, are the major etiological agents of human brucellosis.[5] Contact with sick animals or their fluids, inhalation of infectious aerosols, and consumption of infected animal products like meat and milk are all ways that the disease is passed from animals to humans.[6] It is a risk to those who work with animals, particularly veterinarians, scientists, lab technicians, abattoir employees, and farmers.[7] In humans, the most common symptoms of brucellosis are intermittent fever, fatigue, body aches, joint pains, back aches, chills anorexia, shivering and weakness. Complications like spondylitis, acute respiratory distress syndrome, meningitis, pericarditis, bronchopneumonia, unilateral epididymo-orchitis, wedge-shaped vertebral collapse and uveitis can be can occur.[8] Effective human brucellosis prevention requires the eradication of contaminated animals, immunization of healthy ones, avoiding intake of raw milk, and adequate heat treatment of raw milk.[9] The frequency of Brucellosis in sub-Saharan Africa is underreported and varies by nation, location, and animal variables. Brucellosis is extensively documented in Uganda.[9] Most people in Uganda's cattle-keeping regions depend largely on animals for their livelihood.[10] In Uganda, it's thought that 92% of the raw milk is sold in unofficial marketplaces.

The study was done to determine prevalence of Brucellosis among febrile patients attending Kampala International University Teaching Hospital.

Methods And Materials

Study design

A cross sectional study design was carried out from May to July, 2023 at Kampala International University-Teaching Hospital where febrile patients were selected and enrolled into the study.

Study area

Kampala International University Teaching Hospital is located in Ishaka, Bushenyi District South-western Uganda along Mbarara-Kasese highway, approximately 330 kilometres by road, southwest of Kampala, Uganda's capital city.

Study population

Only febrile patients 5yrs and above attending Kampala International University Teaching Hospital.

Inclusion criteria

1. All febrile patients 5 years and above who consented.

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Exclusion criteria

1. All patients below 5 years
2. All patients who were not clinically suspicious of Brucellosis
3. All patients who did not consent

Sample size determination

Sample size was calculated using the standard formula below:

$n=Z^2P\ (1-P)\ /d^2$ [11]

Where;

n = the minimum sample size required for very large population

Z = the critical value for a given confidence interval

P = expected proportion of the event to be studied

d = margin of error (the margin of error is 0.05)

According to Migisha *et al*. [12]. An estimated seroprevalence rate of 14.9% at 95% confidence interval with a precision of 5% was used. Using the above formula, sample size was;

$n=Z^2P\ (1-P)\ /\ d^2$

$Z^2P\ (1-P)$ =1.96×1.96×0.149×0.851=0.487

d^2=0.025

$n=Z^2P\ (1-P)\ /\ d^2$=0.487/0.0025=195

Therefore 195 participants were enrolled in the study

Sampling technique

Purposive sampling technique were used to select participants into the study where only those patients with history of fever and two or more features suggestive of Brucellosis were enrolled in the study. Febrile patients were conveniently selected and informed on the procedures to be done on them and then requested to fill consent forms. 10mls of intravenous blood were aseptically collected, then 2mls was inoculated into 20mls Brain Heart Infusion broth and then immediately taken to the Microbiology Laboratory for incubation.

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Laboratory methods

Laboratory procedures that were done include blood culture, Biochemical tests and antibiotic susceptibility profile of the isolates.

Blood culture

Sample collection and inoculation

Materials required

- 2% chlorhexidine in 70% alcohol for cleaning of venepuncture site
- Brain Heart Infusion broth
- A needle and syringe
- Gloves

Procedure

Step 1. Skin preparation

- Hands were washed with soap & water
- A tourniquet was applied and area palpated to identify vein
- The venepuncture site was cleaned with a 2% chlorhexidine in 70% isopropyl alcohol swab and allowed to dry

Step 3. Blood collection

- Hands were washed again and dried and then were put on clean examination gloves
- Using aseptic technique, I attached needle to syringe
- The needle was then inserted into prepared vein and 10 mL blood collected in syringe
- Needle was then withdrawn after collecting 10 mL blood in syringe
- 2mls of Blood was then Inoculated into 20mls of Brain Heart infusion broth
- Mixture was then mixed well

Step 4. Patient skin care

- Gauze pad was placed over the site, continuing mild pressure
- When bleeding has ceased, an adhesive or gauze bandage was applied over the site
- After the specimen have been collected, remaining skin antiseptic was removed from collection site using a sterile alcohol swab.

Step 5. Labelling of Brain Heart Infusion bottles

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- All Brain Heart Infusion bottles were labelled

Step 6. Disposal

- The blood collection devices were disposed of in the nearest sharps container according to regulations.
- All other used materials were disposed of in appropriate container and hands washed

Incubation and monitoring

The Brain Heart Infusion bottles were incubated at 37°C aerobically with 5% carbondioxide and then monitored daily for growth for a minimum of 4 weeks for bottles that showed no growth and blind subculturing was done every 7 days.

Subculturing on Chocolate and Blood agar

Subculturing was done on Chocolate and Blood agar in a Class II BSC.

Preparation of Blood agar

- 40grams of blood agar base grams was suspended in 1000 mls distilled water
- Then was heated to dissolve the medium completely
- Then was Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes
- Then was Cooled to 50°C and then 5% v/v Sheep blood was added
- Then was poured into sterile Petri plates

Procedure

- Using a sterile wire loop, Broth was inoculated on to the surface of the media

The agar plate was incubated at 37°C in 5% carbondioxide for 72 hours

Preparation of Chocolate agar

- 40grams of blood agar base grams was suspended in 1000 mls distilled water
- Then was heated to dissolve the medium completely
- Then was Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes
- Then was Cooled to 50°C and then 5% v/v Sheep blood was added
- The medium was then heated at 80°C in a water bath until it turned chocolate
- Then was poured into sterile Petri plates

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Procedure

- Using a sterile wire loop, Broth was inoculated on to the surface of the media
- The agar plate was incubated at 37°C in 5% carbondioxide for 72 hours

Suspect colonies were picked for biochemical testing and gram stain using sterile wire loops.

Gram staining technique:

Procedure:

- Suspect colony was picked using a sterile wire loop and a smear was made on a slide
- The smear was then dried
- The smear was then flooded with crystal violet for 30seconds
- The excess stain was then washed away using tap water
- The smear was then flooded with Gram's Iodine for 30seconds
- The excess Iodine was washed away using tap water
- The smear was then decolourised rapidly using acetone alcohol for 3 seconds
- The acetone alcohol was then washed away using tap water
- The smear was then flooded with neutral red for 30seconds
- The excess stain was then removed using tap water
- The smear was then dried
- The smear was then examined microscopically under x100 objective for gram staining characteristics

Interpretation:

- Organism was either Gram positive (purple/blue couloured organisms) or Gram negative (red/pink coloured organisms)

Biochemical tests

Biochemical tests such as oxidase, catalase, urease, reaction with anti- A and M antisera were done in a Class II BSC to identify the isolates.

Oxidase test

Procedure:

- A commercially available oxidase paper containing the reagent was obtained
- Isolated colony to be tested was picked and rubbed on the paper.
- Colour change was observed within 10 seconds.

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Catalase test

Procedure;

- 5 drops of 3% H_2O_2 was added to a test tube.
- Using a wooden applicator stick, a small number of organisms from a well-isolated 24-hour colony was collected and placed into the test tube.
- The tube was placed against a dark background and observed for immediate bubbles

Urease test

Procedure:

- surface of a urea agar slant was streaked with a portion of a well-isolated colony
- The cap was left on loosely and then incubated at 37° C in 5% carbondioxide for 24 hours.
- change of color was observed in the inoculated medium

Agglutination with anti A and anti M antiseras

Procedure:

- Heavy suspension of the isolates was made on slides in a Class II BSC.
- Then was reacted with anti A and anti M antiseras in a Class II BSC.
- Based on the results of the above immunological results, the isolates were identified accordingly.

Antibiotics susceptibility profile of the Brucella isolates

Modified Kirby-Bauer method and commonly used antibiotics in the management of Brucellosis in Uganda were used. Antibiotics used include Gentamycin (10µg), Doxycycline (30µg), Streptomycin (10µg), Ciprofloxacin (5µg), Rifampicin (5µg) and Cotrimoxazole (25µg). All the antibiotic sensitivity tests were done in a Class II BSC.

Modified Kirby-Bauer method

Materials and reagents needed included Mueller Hinton agar, petri dishes, sterile cotton swabs and turbidity standard.

Preparation of Mueller Hinton agar

- 38g of Mueller Hinton agar powder was suspended in 1L of distilled water.
- Then mixed well and dissolved completely.

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- Then sterilized by autoclaving at 121°C for 15 minutes.
- The medium was then cooled to 50ºC
- 5% sheep blood was then added to the Mueller-Hinton agar and 25mls of the media poured into plates
- Then was allowed to set on a levelled surface
- When the agar solidified, plates were dried for immediate use for 30 minutes at 36ºC by placing them in an upright position in the incubator with the lids tilted.

Preparation of Turbidity standard

- Turbidity standard was prepared by pouring 0.6 ml of a 1% (10 gm/L) solution of barium chloride dihydrate into a 100-ml graduated cylinder filling to 100 ml with 1% (10 ml/L) sulphuric acid
- The turbidity standard solution was placed in a tube identical to the one used for the broth sample
- When not in use, it was stored in the dark at room temperature for six months

Procedure

- Antimicrobial disks were allowed to reach room temperature before opening the container. Using McFarland turbidity standard, a suspension of the test organism in sterile distilled water equivalent to a 0.5 McFarland standard was prepared for each of the isolates
- The suspension was spread onto separate Muller-Hinton agar plates
- Using forceps, selected antimicrobial disks was applied on to the agar
- The disks were placed with an equal distance apart from each other
- After incubation for 48hours at 37°C in the presence of 5% CO_2, the diameter of each zone(including the diameter of the disc) was measured and recorded in mm
- The measurements were made with a ruler on the under surface of the plate without opening the lid.
- The results were interpreted according to the CLSI guidelines for antibiotic susceptibility testing for fastidious organisms and since Brucella specific guidelines are not yet established break points for *Heamophilus influenza* which have similar characteristics with Brucella were used.

Data analysis and presentation

The obtained data was fed into excel spread sheets and then exported to SPSS version 20 for analysis. Descriptive statistics such as frequencies and percentages for prevalence.

Ethical consideration

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Ethical approval was obtained from Institutional Research Ethics Committee of Kampala International University as well as Kampala International University-Teaching Hospital management. Informed consent of the participants was obtained from them before enrolling them in the study. Informed consents for children below 18years was taken from their parents or guardians and assent was taken from children above 7years.

Results

Of the 195 febrile participants sampled, 5 (2.6%) tested positive for Brucellosis while the remaining 190 (97.4%) tested negative for the disease as illustrated in figure 1 below. Prevalence was higher in males 3(60%), highest among age group of 20 – 29yrs 3(60%), highest in the married people 3(60%), Banyankole 4(80%), Christians 4(80%), Farmers 3(60%) and People with Tertiary Education and above 3(60%) as illustrated in Table 1 below.

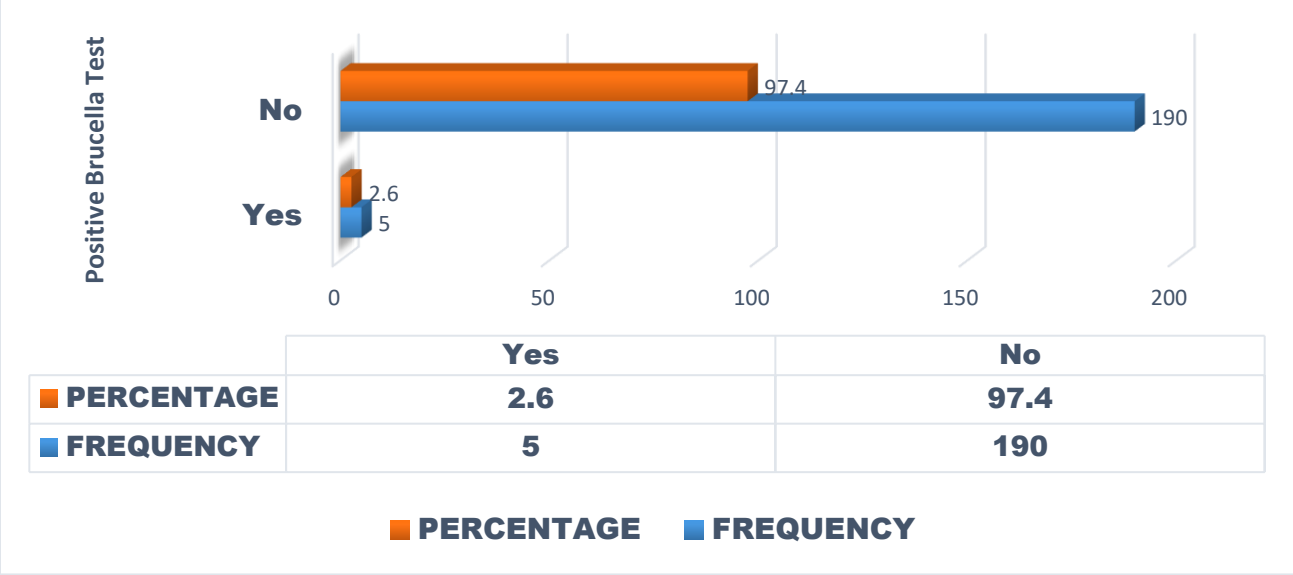

Figure 1: Showing Prevalence of Brucellosis among Febrile Patients at KIU-TH

Table 1: Showing prevalence of Brucellosis among participants

VARIABLE	BRUCELLOSIS	
PARAMETER	+ve, n (%)	-ve, n (%)

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Gender		
Female	2 (40.0)	92 (48.4)
Male	3 (60.0)	98 (51.6)
Age		
< 20 years	0 (0.0)	32 (16.8)
20 – 29	3 (60.0)	58 (30.5)
30 – 39	1 (20.0)	24 (12.6)
40 – 49	1 (20.0)	23 (12.1)
50 years and older	0 (0.0)	53 (27.9)
Marital Status		
Single	2 (40.0)	53 (27.9)
Married	3 (60.0)	121 (63.7)
Widow/Widower	0 (0.0)	6 (3.2)
Divorced	0 (0.0)	10 (5.3)
Tribe		
Munyankole	4 (80.0)	140 (73.7)
Mukiga	0 (0.0)	3 (1.6)
Others	1 (20.0)	47 (24.7)
Religion		
Christian	4 (80.0)	147 (77.4)
Moslem	1 (20.0)	28 (14.7)
Others	0 (0.0)	15 (7.9)
Occupation		
Farmer	3 (60.0)	104 (54.7)
Butcher	1 (20.0)	1 (0.5)
Others	1 (20.0)	85 (44.7)

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Education Level		
None	0 (0.0)	44 (23.2)
Primary	0 (0.0)	93 (48.9)
Secondary	2 (40.0)	37 (19.5)
Tertiary and above	3 (60.0)	16 (8.4)
Had knowledge about Brucellosis		
Yes	4 (80.0)	164 (86.3)
No	1 (20.0)	26 (13.7)
Keep Animals Home		
Yes	4 (80.0)	39 (20.5)
No	1 (20.0)	151 (79.5)
Type of Animals		
Cattle	3 (60.0)	29 (15.3)
Dogs	1 (20.0)	3 (1.6)
Goats	0 (0.0)	3 (1.6)
Sheep	0 (0.0)	1 (0.5)
Pigs	0 (0.0)	3 (1.6)
None	1 (20.0)	151 (79.5)
Drink Raw Milk		
Yes	3 (60.0)	5 (2.6)
No	2 (40.0)	185 (97.4)
Do Milking		
Yes	4 (80.0)	8 (4.2)
No	1 (20.0)	182 (95.8)
Share Water with animals		
Yes	1 (20.0)	6 (3.2)

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No	4 (80.0)	184 (96.8)

Discussion

The study showed that prevalence of Brucellosis among febrile patients was low (2.6%). The low prevalence is attributed to the fact that majority of the participants were not exposed to the infection since most of them reported not to drink raw milk 187(95.9%) and not to practice milking 183(93.8%). This was consistent with reports from a study that indicated a prevalence of 4.3% among febrile patients attending Rushere community Hospital in Kiruhura district, South-western Uganda.[12] Results from the study were also in agreement with reports from a study that showed a low prevalence of 2.9% among febrile patients in Kilimanjaro region of Tanzania.[13] Findings from the study were also similar to findings from a study that revealed a low prevalence of 3.5% among hospitalized febrile patients in Northern Tanzania [14]. However findings were in disagreement with results from a study that indicated a high prevalence of 18.7% in fever patients in post conflict Northern Uganda.[15] This can be explained by the fact that Blood culture method was used which captures only acute cases compared to the Rose Bengal Plate assay that was used in Muloki's study. The Rose Bengal Plate Assay captures both acute and chronic cases.[16] Findings from the study were also in contrast to results from a study that showed a high seroprevalenve of 14.8 among febrile patients in Kiruhura district South-Western Uganda.[12] This is due to the differences in the specifities of the methods that were used. The Blood culture method that was used is highly specific and is not associated with any false positive compared to the Rose Bengal Plate Assay that was employed in Ezama's study.

Conclusion

Prevalence of Brucellosis was low. Brucellosis can exist in isolation or with any comorbidity however it is not associated with any comorbidity.

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