International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 3 Issue 8, July 2016

ISSN: 2394-4404

Prevalence Of Brucellosis As A Zoonotic Disease; A Case Study In Eldoret Municipality-Kenya

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Abstract: ***The Problem statement was to establish the prevalence of brucellosis among the people living within the Eldoret municipality.The objectives of the research were to find out the most virulent species causing brucellosis, the most vulnerable age group, the most effective antibiotic to be used and also to compare the prevalence of brucellosis among human beings and various livestock.The project design was a laboratory based experiments using antibody-antigen serological agglutination methods namely; milk ring test; coombs test; complement test besides others Setting of the research was at Eldoret Municipality slaughter house and at Eldoret polytechnic laboratories. A total of 410 specimens of animal and of human beings were screened for positivity of brucellosis, 135 animal specimens and 135 human specimens were found to be positive for Abortus and melitensis, this information was obtained from the records as well as from the various tests done.From the table No.1 it was noted that the porcine was the least consumed animal by humans thus a decreased number of samples collected leading to a low rate of infection. Table two showed that an equal number of samples were obtained from all the age groups.From the table three Brucella abortus was found to be more prevalent in bovine while the caprine were most infected by the Brucella melitensis both with a prevalence percentage rate of 46% and 31% respectively.Table 4 showed that the most suitable test procedure was the standard agglutination test followed by compliment fixation test; while table 5 showed that Brucella melitensis was the most vilurent affecting the humans while the 21-31 age groups was the most affected group. Milk handlers like the milk hawkers were the most affected occupational group while the veterinary workers were the least affected.***

In conclusion brucellosis was found to be more prevalent in the age group between 21-31 years of age. Brucella malitensis is the most virulent species causing human brucellosis, unpasteurized milk was the most current known source of infection of human brucellosis, while gentamycin and chloromphenical are the most sensitive antibiotics for treatment of brucellosis.It is then recommended that milk should always be pasteurized. Bulls should be castrated and artificial insemination put into practice. Animals should not be allowed to live in the same room with the human beings. Regular inspection of meat and eating joints, dressing of all cuts before handling animal products should be encouraged.

Keywords: Brucella; melitensis; abortus; brucellosis

I. INTRODUCTION

Brucellosis is a disease caused by *Brucella* bacteria. The bacterium is coccobacillary in shape and gram-negative in staining. The Genus Brucella has six species namely *Brucella melitensis; Brucella abortus; Brucella suis; Brucella canis; Brucella neotomae* and *Brucella ovis*. It is a worldwide infection traditionally associated with particular occupations like farming, veterinarians and persons whose occupation involves milk and meat handling. Brucella bacteria are excreted in the milk of infected animals and can be transmitted to man by the ingestion of unpasteurized milk or dairy products such as cheese, butter, yoghurt and ice cream. (Baron; 1982)

In E. Africa, in areas inhabited by pastoralist tribes, brucellosis is fairly prevalent. It only takes a foreigner to accept a drink of pasteurized milk in a local homestead to trigger a new infection Secondly. The bacteria can he passed to man through skin abrasions from directly handling of infected meat carcasses, manure or products of livestock

ISSN: 2394-4404

abortions (Waghela, et. al.,1978).The disease symptoms includes intermittent fever, headache, sweating; chill malaise body aches and anorexia. Eight days are usually needed to diagnose the disease incubation period is 5-6 days but can be even longer.

The disease is incapacitating rather than killing. Diagnosis is mainly made on the basis of clinical symptoms together with a suitable diagnostic test like complement test; antihuman globulin test, milk ring test, blood cultures and even bone marrow cultures. Vaccine is not available attenuated live vaccine for livestock are not attenuated in man and occasional infection of large animal veterinarians comes from the vaccine .Dressing of all cuts and pasteurization of milk should be encouraged. (WHO 1964)

The problem which led to the study was occasioned by the Mating of health animals with already infected animals thus making the disease more prevalent and the handling of infected animal wastes and meat in the slaughter house and Unhygienic hawking and contamination of milk by the farmers in the milking sheds.The study therefore intended to find out the most virulent and common species causing brucellosis in man and animals and the most predominant route of infection of brucellosis and also to determine the most affected age group.

With the increasing number of milk hawkers the unhygienic standards in most of the dairy farms coupled with the ever unacceptable conditions in the municipal slaughter house it has been found necessary to conduct a research on the prevalence of brucellosis within the town and thus recommend to the health public inspectorate department in the municipality on what ought to be done to reduce the incidence of Brucellosis. Kang'ethe *et al.*,2000

B. melitensis was isolated by Bruce in 1 887 from the spleen of tissue of patients who died of Malta fever. Sheep and goats are the preferred hosts of B. melitensis, but other animals may also be infected. *B. melitensis* tends to localize in the reticuloendothelial system and the pregnant female typically results in abortion. Humans are susceptible to infection by .*B. melitensis* which is widely distributed throughout the world but is particularly common in countries around the Mediterranean littoral, the Arabian peninsula, central Asia and parts of Latin America. It probably accounts for majority of cases of brucellosis in humans. *B. abortus* was isolated by Bang in 1897,from cows with contagious abortion and through a series of experiments which demonstrated its specific role in the disease. Cattle are the referred natural hosts of this organism but it can also infect other animals. Its less virulent than B. meletensis, it can also cause brucellosis in humans. (Baron 1982)

B. abortus is more spread than B. melitensis and at one time it has been isolated in virtually all cattle raising countries of the world. Control campaign's have dramatically reduced the prevalence of 13, abortus infection in most developed countries and eliminated it altogether from some of this countries includes Australia, Canada, Czech republic, Germany, Holland, Japan, The Scandinavian countries and New Zealand. It is now uncommon in the USA and most countries of West Europe.

B. suis was first recorded in 1914 by Traum, who reported its isolation from the loetus of the cow. it is a natural parasite of pigs, frequently producing a generalized infection hut with a tendency to localize in the genital tract infection and can also be transmitted to other animals, although the host range tends to be narrower than that of B abortus and B.meiitensis. Possibly for geographical rather than biological reasons. B.ovis was first observed at about the same time.

B.neotomae was isolated by stoenn.er and lackman (9157) from desert wood rats in a remote area of Utah, USA. It has not been associated with disease in humans or the other species and further isolation have not been recorded, the sixth member of the genus is B, Canis which was reported by Carmichael and Bruner in 1 968 as the cause of abortion among the aged dogs in the USA.

it has since been found in dogs of various breeds in many countries including Argentina, Brazil, China, The Czech Republic, Germany, Japan, Madagascar, Mexico, Peru, Papua 1'exv Guinea, and the Philippines. Occasional causes have been reported in humans usually presenting as a mild pyrexial illness (Baron 1982).

Recently, Brucella strains have been isolated from marine mammals (Baron 1982). The isolated compromise at least 2 distinct biogroups corresponding to strains of cetacean and pocine origin. Within these groups there is some variation in metabolic and antigenic properties. However, it is apparent these isolated differ from the Brucella strains infecting terrestrial mammals, they appear to have low pathogenelity for ruminants but circumstantial evidence suggests that they are pathogenic for human.

The genuine Brucella diseases in human and animals infection in pregnant animals particularly cows leads to abortion and involvement of the mammary glands may cause the Brucella organisms to be excreted in the milk for many months or even years. Each of the three main species are pathogenic to man. The incubation period is variable, usually about 10 to 30 days but sometimes symptoms are delayed for several months. infections with the organisms may get localized in various parts of the reticuia-endothecial system, liver, spleen, bone etc with formation of granulomatons lesions resulting in a variety of complications that may involve any other part of the body. Chronic hypersensitivity conditions may develop associated with a long continued illness with vague symptoms of malaise, low-grade fever, irritability and. swelling of joints. (Muriuki *et.al.*,1997)

There are three main species of Brucella that differs in their choice of animal host, in certain cultural and biological characteristics and in the amount of two main antigens that are common to all three species. Br. Melitensis which infects goats and sheep, Br, abortus which affects cattle and Br, Suis which infects pigs. Within the species, strains that differ in some respects from the normal prototypes occur and are considered to be biotypes. There are at least nine biotypes of Br abortus three of Br. Melitensis and four of Br. Suis. A biotype of B ovis which is mainly responsible for testicular infection of rams in various parts of the world including Australia and New Zealand.

All strains of Brucella grow best in a medium enriched with animal serum and glucose and their optimum temperature is 37^0c and the colonies take several days to appear and are normally smooth, moist, transparent and glistening, about 1 mm in diameter (Muriuki *et.al.*,1997)

ISSN: 2394-4404

Tetracycline, or a combination of tetracycline and. streptomycin, provides effective treatment of acute brucelllosis in human; regimens of 2weekiong courses of drug therapy with intervals of 2 weeks between treatments .have been successful, Patients with chronic brucellosis frequently need symptomatic and their response to antibiotics treatment is often disappointing (Baron 1986).

Pasteurization of milk eliminates the risk of brucella infection arising as a result of the consumption of infected milk or milk products. However there remains the possibility of infection due to contact with infected cattle or their tissues so that veterinarians and farmers are particularly at risk (WHO 1964).

Vaccination of cattle between six and eight months of age protects them from abortion due to invasion of the uterus during the first and subsequent pregnancies. However, because of undesirable side effects and the possibility that hypersensitity to Brucella protein may develop vaccination of man is not recommended (WHO 1964).Prevention of brucellosis in man and domestic animals depends on the eradication of the infection from animals by a policy of testing the animals and slaughtering positive reactors (WHO 1964).

II. METHODS AND MATERIALS

Standard methods of examination namely; Serology; Biochemical Reaction; Rose Bengal Plate Test (RBPT); Standard Agglutination Test (SAT); Standard Agglutination Test (SAT); Complement Fixation Test (CFT); Coomb's Test; Milk Ring Test; The Whey Agglutination Test and blood culture methods were all used to determine the presence and absence of the brucella organisms

III. MATERIAL AND APPARATUS

A. APPARATUS

Item	Description
1. Cabinet hood	Size, 460 x 330 x 300 urn CAT No. YXA-600-v
2. Petri dishes	Size 50 x l3urn
3. Incubator	(Economy incubator) Temperature range 5°C to 100°C. 150w max. 480 x 300 mm.
Top Loading Balance	Bosch P.E 622 MAX I 500d = 0.ig
Centrifuge plus	(6 placer 15 mis) CAT
Centrifuge tubes	OCFC-490-C
Dry Oven	200oC. 220 - 240 volts 100 litre capacity
Refrigerator	Lee. British made
Anaerobic jar	for .10 plates
Water bath	Fuse 5 A1P 1 000VA Britain made
Materials	CAT No, Bill 45R-110 x 220 240V
Inoculating loop	Nickel chromium wire CAT No. 24 Swa ECW- 440- 1 70F
Microscope (Compound)	07CAT-No-75F-5243 ON Illuminator 240V 20W
Autoclave	Capacity 48 litre 7 x 4 150- 0109
Measuring Cylinder	Capacity 1000 m/s Pyrex CAT No. Cyl.290-50W

B. MATERIALS

Items	Description
Fibrinated blood	20 ml
Milk	(Unpasteurized)
Blood	serum
Distilled water	1 litre
Blood agar base	51 gms
Cotton wool	Absorbent cotton wool 400g
Surgical gloves	Size 6
Syringes	1 x 100 NORM-JECTr 5 ml made in Germany Cert. DIN/EN/ISO 9001
Needle	Made in Germany, Size: 23G x 1"
Disposable Applicator Sticks	Wooden
Pasteur Pipettes	Disposable length approximately 230 mm.
Methylated Spirit	
Reagents	Br. Melitensis and. abortus.

IV. SAMPLING TECHNIQUES

Blood specimens were collected from different patients suspected to have characteristic fever of brucellosis. The specimen was collected from the left hand vein of the patients using a sterile 5CC syringe and 21 gauge needles.

The blood was transferred from the syringe into sterile specimen bottles. Before the blood specimen was transferred into the centrifuge tubes and the needles were safely removed from the syringe and then the blood was carefully transferred into the specimen containers. The idea of removing the needle first was ideal because if the blood was squeezed directly into the tubes through the needle then the blood would haemolyse hence destroying the red blood cells, The blood was then put into centrifuge tubes and then centrifuged for 5 minutes at 3000 rpm in order to separate the serum from the blood cells. After the separation of the two serum was then pipetted into a different tube and labeled according to the patients laboratory number using a grease pencil.

The reagents kit in the refrigerator was then removed; the reagent kit contained the Brucella abortus reagent and Brucella Melitensis reagents.

The serum containing no traces of blood was then pipetted into a clear card in form of two separate drops and each drop labeled as B.abortus and B.melitensis respectively. The reagents were then dispensed drop wise into each corresponding drop of the serum according to the label on the card and then mixed using an applicator stick and then rocking them non stop for eight minutes.

The reagents contained the antibodies specific for the brucella antigens.

The mixing was done in a circular and steady motion and a lot of care was observed not to contaminate one well with another hence produce false results.

The results of the wells were reported by presence of agglutination and absence of agglutination.

Finally the results were interpreted as either positive in the presence of agglutination and negative test in the absence of agglutination. The results were then recorded in a register and the positive seras were kept for confirmation. The test card was then dipped into a sink to await cleaning.

From animals, blood was drawn from the jugular veins using a 10cc syringe with 10 gauge needle apart from the pigs (porcine) where the tarsal vein was used.

A sterile bottle of approximately 10 ml was used to carry milk from the dairy farms or from the milk collection centres to the laboratory. Milk for MRT i.e. milk ring test was usually preferred to be fresh only. For the culture of the animal specimens in the isolation of the brucella species the placenta or placental materials were obtained from the foetal stomach contents, liver and kidney organs were also used for culture (Waghela, and Karstad, 1986).

V. EXPERIMENTAL PROCEDURES

BRUCELLA SERUM AGGLUTINATION TEST (SAT)

- ✓ Eight serological tubes were arranged in two rows and
- ✓ labelled as Bal, BA2, BA3, BA4, BA5, BA6, BA7,BA8 in the first row.
- ✓ The tubes in the second row were labelled as Bm 1, Bm2, Bm3, Bm4, Bm5, Bm6, Bm7, Bm8,
- ✓ A pasture pipette was used to dispense 1.9 ml of normal saline into tubes No. 1 both in BA and Bm and lml of normal saline into each of the remaining 7 tubes in both rows. 0.1 ml of the patients serum was dispensed into the first tubes in both BA and .BM to make a dilution of 1:10; this was mixed properly and 1 ml of the mixture was transferred into the next tubes i.e. the 2 tube up to the last tube then the 1 ml the last tube after mixing was discarded; this was repeated for the 21 row.
- ✓ One drop of the Abortus reagent was added to all the tubes labeled BA and one drop of the Melitensis reagent was added to all the tubes labelled BM. All this tubes in both rows were mixed and incubated at 37°C for 4 hours, (Waghela and Karstad 1986).

INTERPRETATION OF THE RESULTS

- ✓ The titre was taken at the last tube to show macroscopic agglutination.
- ✓ Titre equal or greater than 1:80 was considered clinically significant hence brucella test positive.

RESULTS

Tube	1	2	3	4	5	6	7	8
Dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Titre	10	20	40	80	160	320	640	1280

ROSE BENGAL PLATE TEST (RB.PT)

This was a qualitative test for the brucella antibodies which employed the use of a white tile which was divided into two parts and marked A and M respectively.

One drop of the patients serum was added in both parts marked A and M. One drop of Abortus antigen suspension was added to part marked A and Melitensis antigen suspension to part marked Al and then all this were mixed by using different applicator sticks and reaction inform of agglutination was observed within 60 seconds or 1 minute after complete and continuous rocking of the tile for one minute. if no agglutination the test was reported as negative but the presence of agglutination was a positive result hence a tube method was desired for confirmation.

BRUCELLA MILK RING TEST (MRT)

A drop of milk was put into a 75 x 8.5mm column test tube. Then 0.03 ml of antigen was added to each milk sample.

A Known positive and negative samples were include as control. AU the samples were thoroughly mixed by jaki.ng up the samples and then incubated atw 37°c, this was then followed by putting them into a water bath for one hour. The results were tabulated as follows.

Cream ring	Milky	Rating
Deep blue colour	White	++++
Define colour	Slight colour	++
Creaming and milky	Moderate colour	++
Define color	same colour	+
White or slight colour	Different colour	-ve

It was further noted tat the milk from the goats stained with heamatoxylin and antigen was normally agglutinated and sedimented to the bottom of the tube rather than forming a ring. One drop of sodium chloride was added to the milk to eradicate this problem.

BLOOD CULTURE

- ✓ 10ml of blood was withdrawn from the patient.
- ✓ 5 ml of the blood was added into Culture tubes containing glucose serum broth
- ✓ One tube of each pair was incubated in an atmosphere containing 10% carbon dioxide preferably in an aerobic jar.
- ✓ After three days subcultures were made onto serum-dextrose agar which was a selective media for the brucella species.

Species	Number	%
Bovine	60	28.5
Porcine	30	14.5
Ovine	60	28.5
Caprine	60	28.5
Total	210	100

Table 1: samples of livestock species analyzed from April to December 2008

SAMPLES OF LIVESTOCK'S SPECIES ANALYZED FOR SIX MONTHS

Age group	Male	Female	%
10-20	20	20	20
21-31	20	20	20
32-42	20	20	20
43-53	20	20	20
54-64	20	20	20

Tables 2: sample of human beings analyzed in the 6 months

Animal species	Brucella species				%
Species	Abortus	Melitensis	Suis	Total	
Porcine		_	1		1
Ovine	5	15	10	30	22
Caprine	10	31	2	42	31
Bovine	40	15	2	62	46
Total				135	100

Table 3: Brucella species identified in different livestock

Specimen	SAT	RBPT	CFT	TOTAL	%
Caprine	10	5	5	20	14
Bovine	15	5	10	35	24
Ovine	5	2	2	9	6
Porcine	1	_	_	1	11
Human	40	20	20	80	55
Total				145	100

Table 4: positive human and livestock specimens on various tests

Occupation	No positive	No negative	Total	% positive
Meat handler	10	10	20	18
Milk handler	18	2	20	32
Veterinary workers	12	8	20	21
Laboratory personnel	16	4	20	29

Table 5: occupational seropositive of brucellosis among people of different selected occupation at risk

VI. DISCUSSION AND ANALYSIS OF RESULTS

From the table number 1 it was noted that a total of 410 samples of human and livestock animals were analyzed for brucellosis selectively from April — December 2007. During the dry period of the year April — June milk production was very low and the only available milk went to the milk hawkers and the rest to the farmers themselves. As a result, the milk processing plants like the local company of K.C.C and Broke Side did not receive enough milk and thus we had a shortage of pasteurized and safe milk. This actually made the farmers and the hawkers to enjoy a lot of monopoly since they were the only source of supply to the consumers. And as the farmers and hawkers were enjoying this trade the disease progress was reported to be increasing.

The milk alone was found to have the highest incidence and this was actually related with the increased number of hawkers of the commodity who normally subject the milk to a lot of contamination besides the consumers who were reported not to be pasteurizing the milk effectively. This increased incidence was also related with the decreased vegetables in the market because most people especially from Kamkunji and Bondeni and even some from Huruma and Langas had now started casual jobs in the Eldoret slaughter house whereby they acted as transporters of the already skinned animals and also participating in the cleaning of the intestines of the slaughtered animals.

The people instead of washing their hands clean before leaving for their meals which they took just in the nearby Kiosk went straight to the Kiosk and started eating with the contaminated hands hence increasing the spread of this disease especially the Bovine type because cattle were being slaughtered in large numbers as compared to other animals. The study also revealed that most people of the studied areas got the disease from the eating places which were all unhygienic as the studied areas were all slums. From the table 4 it was also noted that the most sensitive test for this disease was SAT followed by the CFT (compliment Fixation Test). This method of testing was found to be reliable and effective because even in the slightest presence of the bacteria the test method was detecting very much accurately.

The result in table 5 showed that the *brucella militensis* was the most virulent species affecting man as compared to other species.

Brucella abortus and *Bruella meiltensis* were found to be the most virulent species affecting cattle and goats respectively as shown in table three.

.Brucellosis is more prevalent among human indicated in the table No. 5 brucellosis is an occupational disease in most cases and affects the veterinary: workers: laboratory technicians: milk handlers and meat handlers. The table 6 showed that milk handlers were at higher risk of infection as compared to the rest and this was because of the infectious agents found in milk and with which the milk handlers were frequently exposed to. From the study area of Kamukunji, some parts of Huruma and Bondeni slum dwellers, majority of them kept goats (caprine) at the shortest space available with the animals just tied inside their bed rooms. Sample specimens taken in four houses of Kamukunji area were tested for brucellosis and out of the four only one was negative, Brucella abortus and melitensis were isolated from the specimens. The patients from where the melitensis species were isolated from seemed to have serious clinical manifestations as compared to the abortus manifestation cases hence the melitensis was proved to be the most virulent species.

From the study it was also established that the close association with domestic animals through either housing or occupational duties contributed to the spread of this disease. The disease was also being contributed to the spread of this disease.

The disease was also being mistaken for other diseases like typhoid and malaria which are very much common and also share the same symptoms and signs hence poor diagnosis and treatment of brucellosis. The commonest species noted was *B. melitensis* due to the close association of goats and men because of the poor housing conditions in these areas. Out of the 16 cases of the drug sensitivity done only 10 cases responded well by 4 and 6 cases for chloromphenical and gentamycin respectively. Penicillin and streptomycin gave poor results.

ISSN: 2394-4404

VII. CONCLUSION AND RECOMMENDATION

Brucellosis is more prevalent in females than in male and the age of between 21-31 years was the most affected age group. *Brucella melitensis* is the most virulent species of Brucella causing Brucellosis in human beings in the study area. Milk handlers were found to be in high risk of infection as compared to other occupational groups followed by laboratory technicians as shown in table 6.Unpasteurised milk is the most current known source of brucellosis and thus human infections are through milk since the manner of boiling employed by the farmers and customers does not frilly kill the Brucella organisms especially in preparing traditional 'mursik'. Gentamycin and chloramphenical are the most sensitive antibiotics for the brucella species. All milk including goats milk and products should be pasteurized. Infected animals should be removed or isolated so as to prevent further transmission of the disease other animals. Public health act that prohibits any animal being kept within the same room with human beings should be enforced. Since the organisms can enter the body through cuts or abrasions of the skin the people dealing with the animal or human products should dress the cuts properly. Chloromphenical and Gentamycin are the most suitable antibiotics for treatment for brucellosis.

REFERENCES

[1] Baron S, MD (1982) J.A.M Vet. 26, 35
[2] Baron S; 1986: Medical Microbiology; Addison-Wesley publishing company. Inc. Second Edition page 514. (2)
[3] Kang'ethe, E. K., Arimi, S. M., Omore, A. O., McDermott, J. J., Nduhiu, J. G., Macharia J. K. and Githua, A. (2000). The prevalence of antibodies to *brucella abortus* in marketed milk in Kenya and its public health implications. Paper for oral presentation at the 3rd all Africa conference on animal agriculture. 6 – 9 Nov2000.
[4] Muriuki SM, McDermott JJ, Arimi SM, Mugambi JT and Wamola IA (1997). Criteria for better detection of brucellosis in the Narok District of Kenya. *East Afr Med J.* 74(5):317-20.
[5] Waghela, S., Fazil, M. A., Gathuma, J. M. and Kagunya, D. K. (1978). A serological survey of brucellosis in camels in north-eastern province of Kenya. *Trop. Anlm. Hlth Prod.*10:28-29
[6] Waghela, S. and Karstad, L. (1986) Antibodies to *brucella* spp. Among blue wildebeest and african buffalo in Kenya. *Journal of Wildlife Diseases.* 22(2):189-192
[7] WHO, 1964 except committee on brucellosis. World health organization technical report service no. 289.