Research Article

Comparative Prevallance and Pathological Changes on Camel Brucelosis at the Selected Slaughterhouses in Garissa County, Kenya

Abdirahman Barre*, Karanja D Njuguna, Bebora Lilly Caroline and George Chege Gitao

Faculty of Veterinary Medicine, Department of Veterinary Pathology, Microbiology and Parasitology University of Nairobi, Kenya.

Abstract | The study aimed at determining the presence of the disease in camel slaughterhouses in Garissa County, through serological testing and pathological lesions that encountered at post mortem inspection of camel meat. Three sub-counties; Garissa Township, Dadaab and Balambale were purposefully recruited based on presence of camel slaughterhouses and accessibility. A hundred and sixty (160) camels were selected from 238 presented during the visits based on clinical manifestations suggestive of *Brucellosis* obtained upon ante-mortem examination and clinical history from owners. Sero-prevallance determination that involved the blood collection from the jugular and screening serum for attendance of *Brucella* antibodies using Rose Bengal Plate Test (RBPT), serum agglutination test, competitive-enzyme linked immune sorbent assay and double agar gel immunodiffusion test. The selected camels were followed into the slaughterhouse and pathological changes were identified grossly and microscopically based on alteration in organ and/tissue structure. The three main clinical signs that suggested brucellosis were lameness, swollen lymph nodes and abortion. Out of 160 samples tested, 15 (9.37%) were positive for *Brucella* antibodies and evenly distributed between counties; 8% (4/50) for Garissa Township; 10% (5/50) in Dadaab and 10% (6/60) in Balambale. Using chi-square ($\chi 2$), there was no statistically alteration in sensitivity among the four serological tests (p=0.999). Seventy-eight (48.7%) camels had one or more organs with lesions leading to condemnation at meat inspection. The common gross lesions encountered were fibrin depositions 3 (1.8%), enlargement of lung 2 (1.2%), pericarditis 38 (23.7%), and hepatomegaly with nodular liver lesions 79 (49.3%), enteritis 5 (3.1%), haemorrhages and congestion of visceral organs (lung and kidney) 6 (3.7%). Histopathology of sero-reactors revealed; cellular infiltration in lymph node 9 (5.6%), hypoplasia of lymphocytes 6 (3.7%), collapse of alveoli 5 (3.1%), oedema, congestion 4 (2.5%), fatty degeneration in liver 3 (1.8%) and haemorrhages in kidney1 (0.6%). In conclusion, brucellosis is prevalent in camel in Garissa County. Further extensive research should be done in the whole country. With respect to picking positive cases, RBPT is recommended as a screening test, since it is cheap, quick, and easy to carry-out. The other three can be used to establish respective antibody titres. The organs condemned at inspections are due to inflammatory processes that can be associated with brucellosis or other zoonotic diseases. Standard biosecurity measures at slaughterhouses and farms be enhanced the control and prevention of *Brucella* infection to animals and human.

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***Correspondence** | Abdirahman Barre, Faculty of Veterinary Medicine, Department of Veterinary Pathology, Microbiology and Parasitology University of Nairobi, Kenya; **Email:** idaajaaa007@gmail.com, idaajaa007@hotmail.com
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Introduction

Camels are an adaptable animal and has been domesticated by man. It's been the quickest mode of transport in deserts due to that it's also referred to as the ship of desert (Bahrawy *et al*., 2015). It is also used for economic and social aspects; they are used for milk, meat and conceal provide, as for different functions like transport, amusement, celebration and competition as in athletics and wonder show (Kaskous, 2016).

Camels (*Camelus dromedarius*) are foremost livestock in North-Eastern province wherever it offers nourishment to several individuals, particularly throughout the frequent droughts once different animals either die or are unthrifty (Wanjohi *et al*., 2012). This is as a result of the artiodactyl mammal is extremely fitted to hot desert, semi-desert, arid and semi-arid areas. The camels are prevailing domesticated animals in North-Eastern region where it gives sustenance to numerous individuals particularly amid the regular dry spells when different creatures either bite the dust or are unthrifty. Camel populace in Kenya is more than 1 million and about 54% of them are kept in Garissa and Wajir locale. Occupants of these bone-dry regions are for the most part of Somali root and are pastoralists.

Globally, camels are important for industrially and financially, in keeping with the continuing report there are about 600,000 camels in Kenya (According to Food and Agriculture Organization of the United Nations. Almost of all these are kept by migrant pastoralists in the arid lowlands of Northern Kenya. Camel brucellosis has been accounted for in all camel-raising nations. The contaminations are on the ascension in Old World camels (OWCs) because of the uncontrolled exchange of live creatures.

In Kenya, there are three sorts/types of a camel: Turkana (it is little in size; averaging 350 kg), Rendille/Gabbra (300 kg) and Somali (biggest in body estimate; 550 kg). The camels are utilized as multifunctional creatures in the peaceful generation framework. They are great drain makers: Delivering more drain contrasted and dairy cattle and little stock. They, accordingly, prove to be useful amid the dry season; the pastoralists incline toward camel drain to that of other domesticated animals' creatures as a result of its delectable taste and its being nutritious (Kaindi *et al*., 2011).

About 60% of Garissa County population are pastoralists who keep around 300,000 camels that increase the animals' economy of Carissa County. The indigenous camel breeds that found in North Eastern Kenya Are include: Somali breed, Turkana and Gabra. Camel *Brucellosis* is an infectious chronic bacterium, sickness of camel and other species, that mainly caused by individuals from class that influences for various individual species. Therefore, condemned organ (visceral organs) in camel slaughterhouses have been documented around the world. The disease spreads from herd to herd or from animal to animal or also from country to country (Garcell *et al*., 2016).

Brucellosis is furthermost Zoonosis infection in Animals and man. All most all the infections are transmitted from animals to man by drinking uncooked milk, meats as liver and kidneys from effected animal and close contacted of the animals through breathing, slaughtering and contaminated dusts. The illness is caused by bacteria genus called *Brucella*. There are two (2) common species in camel *Brucella melitensis* and *Brucella abortus*. In terms of camel production farms, the sero-prevalence is higher in intensive camel rearing farms while in extensive system farms the incidence is very low.

Organ condemnations have commercial and public health significance associated direct economic losses. Therefore, condition that leading to organ condemnation in camel slaughtered are bacterial and parasitic infections and non in factious organism.

The significant reasons for condemned organ in butcher stock are parasitic disease (*Hydrated cyst* and *Fasciola* spp. in camel) and bacterial diseases (brucellosis, leptospirosis and Tuberculosis).

Materials and Methods

Study area

This study was carried out in Garissa County (Figure 1). The county is one of the three countries in the North Eastern region in Kenya. It is located in Eastern Kenya bordering Somalia at the East, Wajir County and Isiolo County at the North, Tana River County at the West and Lamu County at the South. It lies latitude of 10 58'North and 20 1' South and longitude of 380 34' E and 410 32' E. the county covers an area

of 44,174.1 Km². It is arranged at 0.46° South scope, 39.66° for East longitude and 152 meters height over the ocean level.

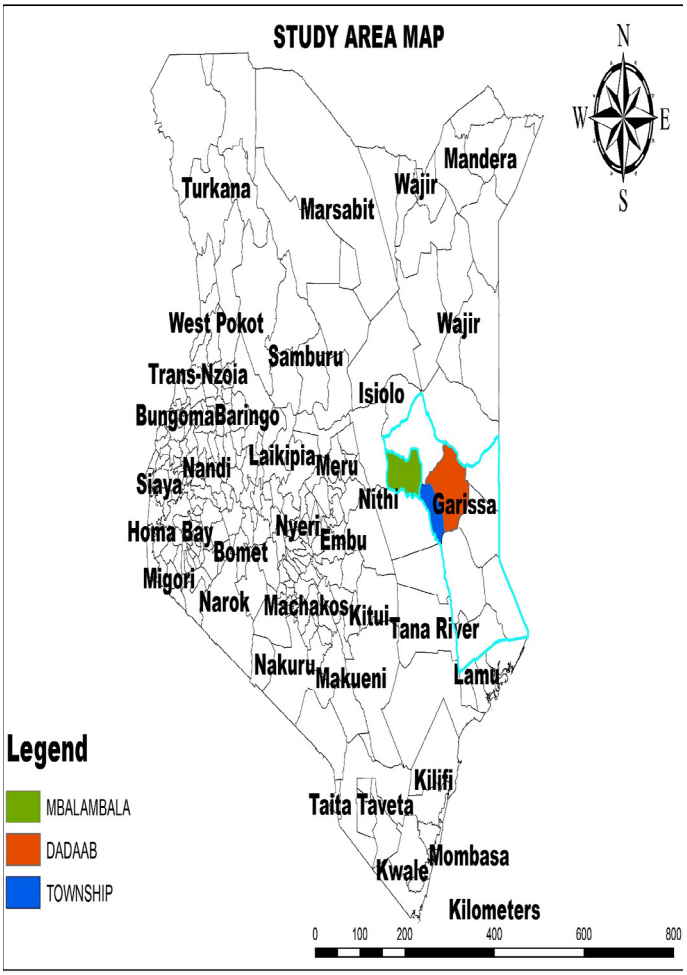

Figure 1: *Map of Kenya showing that three sub counties and County of Garissa (Kenya Political Map, 2016; Kenya National Bureau of Statistics, 2017).*

The agriculture and livestock are pilars of the county economy and they are the maine sources of occupation, livelyhood for farmers and other in the value chaine. The county is flate physiclly and topogaraphically and is low lying without hills, valleys and mountains. The county is principally a semi-arid area falling within ecological zone V-VI and receives an average rainfall of 275 mm per year. There are two rain seasons, the short rains from October to December and the long rains from March to May. The natural aired of the coounty temperatures are generally high throughout the year and range from 20°C to 39°C. The average temperature is however 36°C. The hottest months are September and January to March, while the months of April to August are relatively cooler (Wanjohi *et al*., 2012).

There are twenty (20) camel slaughter facilaties in the county. Six (6) are located in blambale sub-county.five (3) is in dadaab, eight (4) in township, two (2) is in Fafi sub-county, three (3) in lagdera sub-county and two (2) in Ijra sub-county. The others are uncategorized ones and don't operate daily according to the sub-county veterinary officers (SCVO).

Study design

The study was carried out both prospective study (sero-prevalance) and cross-sectional survey (pathological lesions) in different slaughterhouses of Garissa County Kenya which were include Garissa central Sub-county (Garissa Township), Garissa East sub-County (Balambala) and Garissa west sub-county (Dadaab). For Garissa County Kenya, based on the The Avalability of animals (camels) and security. for the sero-prevalance study involved analysis of data from the screaning part of four (4) diferent serology tests. On the other parts of study, cross-sectional study entailed the poste-mortem inspection of the tested RBPT and slughterd carcases and exenterated organs, gross pathological lesions apprent brucellosis and collection of specimens for histopathology analysis.

Selection of slaughterhouses for collection of data

The sub-county Veterinary Officers (SCVOs) in the three study sites were visited to discuss sampling procedure, livestock movement and other necessary assistances. The animal handles at the slaughterhouses were interviewed before sampling. However, the disease under the study were introduced to the slaughterhouse owner and the Veterinary Professional doctor at the slaughter.

The three (3) slaughterhouses namely: Township, Dadaab and Balambale in Garissa-county were selected through convenient sampling methods. They were selected based on the higher number of camel availabilities for slaughter and security compering by the other slaughterhouses of the county and available resource for laboratory (data recording, sample collection, analysis materials and transportation lab material for sampling) and also availability of poste-mortem inspection instrument.

Study animals and sampling methods

All camels presented for slaughter during the times of visit were examined ante-mortem and records reviewed for signs suggestive of brucellosis. The study animals were apparently health and adult of both sexes. The animal details: tag number, species, sex,

breed, age, and owner of the animals were noted and recorded in slaughterhouse interim data capture of sheet (Appendix 7.6). The three (3) slaughterhouses in the sub-counties were convenient selected for the study. This is because they slaughter a large numbered of camels, they are easy to reach and secure. Only slaughterhouses that handle camels were recruited and visited in a period of four (4) weeks.

Sample size determination

The sample size of various camel species and different slaughterhouses from the County of Garissa were bled for prevalence estimation of the brucellosis in camel and pathological lesions of the positive's animals were determined for the following equation.

$$n = \frac{Z\alpha^2 pq}{L^2}$$

Where; n is required sample size; Zα= 1.96 the normal deviate at 5% level of significant; p A priori estimation of prevalence for the disease; q=1-p and Lis allowable error of estimation.

Slaughtered camel: using the highest prevalence estimation of 15% for brucellosis in camel and L is at 5%.

The required sample size ware calculated:

$$n = \frac{1.96^2(0.15)(0.69)}{(0.05)\hat{}2} \quad n = \frac{3.8416\ (0.15)(0.69)}{(0.05)\hat{}2} \quad n = \frac{3.8416(0.15)(0.69)}{0.0025} = 160$$

Therefore, the percentage (%) prevalence of the disease was calculated by dividing at ratio of the total number of positive from different slaughterhouses for 4:4:5 at Township: Dadaab: Balambale respectively, in order to get the required sample size. The study animals were sequentially selected for inspection and slaughtering on the slaughterhouses at a time to reach the total calculated sample size which is one hundred and sixty (160) camels.

Prospective study: Determination of Sero-prevalance in selected camel slaughterhouses in Garissa County Kenya

Sample size was redistributed to the three sub-counties propositioned based on previous in past slaughtered numbers. In the first two weeks Garissa-township sub-county slaughterhouses were visited and blood collected from fifty (50) brucellosis suspected camels. In the next two weeks, Dadaab and Balambale were also visited and Fifty (50) and sixty (60) brucellosis suspect camels, respectively. Ten millilitres (15ml) of blood was collected from jugular vein using gage 18 needle and 20 ml syringe for serology and the animals was followed to slaughter for collection of any condemned organs.

Serum preparation

The blood samples from camel slaughterhouses (160) prevalence were left to stand over-night in cool box with ice to allow the serum separation and clotting at Garissa veterinary office VIL (Veterinary Investigation Lab). The vials were labelled and stored in freezer (-20°C) at veterinary Investigation laboratory office in Garissa County. The serum was separated by using centrifuge and extracted the stander procedure.

Rose bengle plate test (RBPT)

The Rose Bengal test (RBT) was carried out using the method and OIE the antigen having been obtained from Spain (Rose Bengal- Antigen, rapid slide agglutination antigen, verw, blasé, Instituto de Salud de Navarra, RSA-RB:330-04:4000; in diagnostics ID vet 149. Spain). The temperature of the serum samples was raised to room temperature (21°C) before testing. Using micro-titre pipette a drop (25µl) of serum was placed on the glossy side of the tile: it was then mixed with the drop (25µl) of antigen. The tile was then rocked up-and-down for up to 4 minutes. Positive result appeared as pink agglutination, while no agglutination was taken as negative reaction. Positive and negative control were also set-up.

The results were readied by standard period of RBPT Test. After following the protocol, the agglutination denoted a positive test (+ve) while lack of it means a negative (-ve) results negative. The positive and the negative control were used to monitor the performance of procedure and to compere the interpretations.

This test was carried out using the method of Rose Bengal stained *Brucella* antigen having been from Spain (Rose Bengal-Antigen, rapid slide agglutination antigen, verw, blasé, Instituto de Salud de Navarra, RSA-RB: 330-04:4000; in diagnostics ID vet 149. Spain). Test serum was double diluted in micro0titre wells; first placing 90 µl of PBS (Phosphate Buffer Solution) in the first well 50 µl of PBS in the other wells. This was then followed by placing 10 µl of the test serum to the first well; mixed thoroughly, then 50 µl transferred to the next well and mixed thoroughly. The procedure was then repeated, transferring 50 µl of serum-PBS mixture from the second well to the

3rd one; continuing with the transference of 50 µl of thoroughly-mixed serum-PBS mixture to the next well until the last well. A volume of 50 µl was then removed from the last well and discarded. Then to each well, 50 µl of antigen was added, mixed thoroughly and the plate incubated Overnight. The positive result appeared as pinkish matt across the well, while negative reactions (no agglutination) appeared as a button at the button of the well. Positive and negative controls were also set up.

Competitive enzyme linked immuno-sorbent assay (c-ELISA) tests

The competitive enzyme linked immunosorbent assay (c-ELISA) ID screen: Copmelisa 400 A competitions ELISA kit for diagnosis of brucellosis. The reagents were prepared as per instructions of the kit manufacture (Veterinary laboratory of United Kingdom (UK). and test were prepared and carried out department of VPMP faculty of Veterinary Medicine University of Nairobi.

The serum was run in duplicate by using the comparison of the Optical Density (OD) for each sample. The cut-off point of the positive and negative control were calculated by different variable means of Optical Density. For the analysis results the lack of colour were indicated that the sample tested was positive. Appositive/negative cut-off were calculated as 60%of the mean of the optical density (OD) for the four (4) conjugate control wells. Any test sample that giving OD equal to or below the value was regarded as being positive. However, the sero-prevalence of the binding ratio was calculated by:

$$\text{Binding Ratio} = \frac{mean\ of\ 6\ negetive\ control\ wells}{mean\ of\ 6\ posirive\ control\ wells}$$

Table 1: *The interpretation of brucellosis ELISA test results.*

Serum	
Test result	**Rank**
P/R≤40%	Negative
40%<P/R <50%	Doubtful
50%<P/R≤70%	Positive
P.R<70%	Strongly positive

Ager Gel Immuno-diffusion test (AGID)

Slide ager gel double immunodifusion test (AGID) for anti *Brucella* antibody evaluated in this study is based on a single *Brucella* antigen, referred to the other antigens the test has been done in Bacteriology laboratory at University of Nairobi Department of Veterinary Pathology, Microbiology and Parasitology (VPMP). The test was used; following the method using 1.2 mm diameter puncture. Wells were dug into the solidified ager on the slide (6 in periphery and one at the centre); the central well was filled with the test serum while the outer wells were filled with the antigen. The slide was then incubated at room temperature in humid chambers/petri-dish and reading was done after 24 to 48 hours. Therefore, the presence of curved precipitation lines showed positive reaction. Positive and negative control ware also set-up.

Cross-sectional study: Establishing of Pathological lesions apparent from Brucellosis and organ condemnation

All camels that were suspected as brucellosis at ante-mortem examination were followed into the slaughterhouse. At the post-mortem inspection, organs condemned were grossly examined and sampled for histopathology.

Post-mortem examination

Poste-mortem inspection of organs that were positive in RBPT test was carried out as described by visual, observation, palpation and opening of the effected for each organ. From each Slaughterhouse visited, after anti-mortem examination of the animals were followed for further Pathological and histo-pathological examination. The lesions recorded (Appendix 7.7) with a special attention to the various changes in the organ condemnation based on size of the organ, colour, and transactional appearance. The lung, lymph nodes, heart, liver and kidney were grossly examined and processed for histopathology to rule out other diseases rather than *Brucellosis*. The observed lesions were described, for location, distribution, colour, size and recorded for diagnosis. The morphological lesions and other suspected abnormalities were also recorded. The carcase was disposed of in slaughterhouse departmental disposal container after proper disinfectant of all surfaces and materials during post-mortem examination by using (Benzyl, dimethyl, Ammonium-chloride and Cooper manufactured, Kenya). Photographs of the lesions were taken using by a digital camera (sonny CSD–W920 and Mobile Camera A50 having three optical camera Magnification X40, X10, X100 and X400) and transferred into a computer and labelled appropriately.

Sample collection for histopathology

The Sampled tissue from different Slaughtered camel were fixed 10% Formalin for 48 hours as the stander protocol. The fresh tissue was removed as quickly as possible from different slaughterhouses of camel at Garissa, labelled the container and then transported to The University of Nairobi Department of Veterinary Pathology, Microbiology and Parasitology (VPMP). The tissues were trimmed with the thickened of 5 mm and dehydrated for the concentration of Alcohol for 70% to 95% at the intervals of one and half hour (½) by utilizing 80% of ethanol alcohol for 4 hours. They were cleared, infiltration with the liquid paraffin wax (paraplast) at 60 °C in two changed for the three hours per each and embedded in paper with wax, fixed into the wooden block by using hot searing spatula. The tissue was cut in to the 5µm by blocking and microtoming to the specimen. They were dewaxed in each spaceman for 5 minutes. The tissue was rehydrated and putted distilled water for 5 minutes in each section of the specimen.

The section was stained by using haematoxylin and eosin (H and E). The cover slip was applied by DPX (Dibutylphthalate xylene). The sectioned tissue was inspected under light microscope lens utilizing; x4, x10, and x40 amplification then the pathological lesions were recorded according to the affected organs.

Data analysis and presentation

The information (data) was gathered through descriptive examination from the investigation zones, revised composed and organized. So that the obtained data from, serological tests and pathological lesions were recorded in research notebook and entered the spread sheet of (Ms-Excel) and analysed by stata for windows (version 14.0). And also used Chi Square test (X^2) for compering positivity of the disease from selected slaughtered camel through the pathological lesions of the infection to the other suspected diseases.

Results and Discussion

Prospective study: Sero-prevalence study results

In this case the study was based on four (4) different standard serological test so as to compare their results namely: Rose Bangle Plate (RBPT) serum Agglutination Test (SAT), Competitive Enzyme Immuno-Sorbent Test (c-ELISA) and Ager Gel Immuno-difussion Test (AGID). Therefore, using these serum techniques the results were looked at histological analysis to ensure that same sample has lesion for pathology.

Rose Bengal plate test (RBPT)

A one hundred (160) camel serum samples from Garissa sub-counties in selected slaughterhouses were tested using the Rose Bengal Plate Test (RBPT). Fifteen (15) samples (9.3%) tested positive (Table 2). From Garissa-township (n=50) four (4) samples (8.0%) were tested positive, fifty (n=50) samples from dadaab slaughterhouses six (6) (12.0%) were tested positive while sixty (n=60) samples from Balambale slaughterhouses five (5) (8.3%) were tested positive.

Table 2: *Rose Bengal Plate Test (RBPT) results overall and with respect to the three-study area of Garissa County Kenya.*

Slaughterhouse	No. tested	No. positive	% Positive
Overall	160	15	9.3
Garissa township	50	4	8
Dhadhaab	50	6	12
Balambale	60	5	8.3

Serum agglutination test (SAT)

A one hundred and sixty (160) samples of the camel serum from selected slaughterhouses in Garissa sub-counties were tested using the Serum Agglutination Test (SAT). Sixteen (16) samples (10.0%) tested positive. Garissa-township (n= 50), 4 samples (8.0%), were positive while 46 samples (28.75%) tested negative. from Dhadhab District (n= 50) 6 samples (12.0%) which were higher positive and Balambale also had 6 samples (10.0%) were tested positive. Therefore, after the result in SAT compared at percentage level, Dadaab had a highest percentage reactor of the samples (12.0%) than others.

Competitive enzyme linked immunosorbent assay test (cELISA)

The competitive enzyme-linked immunosorbent assay (cELISA) format has proven to be an accurate, reliable, easily standardized, and high-throughput method for detecting the *brucella* antigen in large and small animals. A one hundred sixty (160) serum samples from camel in three sub counties of Garissa County were tested used by the competitive enzyme-linked immunosorbent assay (cELISA).

The test results were as recorded in prospective. Table denotes that the fivty (50) samples were taken to Garissa-township forty-six (46) samples tested were

negative (-Ve) while four (4) samples for (8.0%), were positive(+Ve) fifty (50) samples from Dhadhaab were a tested forty-five (45) tested negative (-Ve) while five (5), samples for (12.0%) tested positive(+Ve). and sixty (60) samples from Balambale were also tested fifty-four (54) samples tested negative (-Ve), while six (6) samples of (8.3%) tested positive (+Ve) dhadhaab was a higher percentage of reactors (12.0%) than garissa-township and balambale (8.0-8.3%). Therefore, representing the Results for the competitive enzyme-linked immunosorbent assay (cELISA) of three Sub-Counties in Garissa Kenya. The different of the results were shown below Table 4 written by the positive and the negative number in sub-counties as dhadhaab is much prevalence then the other.

Table 3: *Serum Agglutination Test (SAT) results overall and with respect to the three study areas of Garissa County, Kenya.*

Slaughterhouse	No. tested	No. positive	% Positive
Overall	160	16	10
Garissa township	50	4	8
Dhadhaab	50	6	12
Balambale	60	6	10

Table 4: *Competitive enzyme-linked immunosorbent assay (c-ELISA) test results overall and with respect to the three study areas of Garissa County, Kenya.*

Slaughterhouse	No. tested	No. positive	% Positive
Overall	160	15	9.3
Garissa township	50	4	8
Dhadhaab	50	6	12
Balambale	60	5	8.3

Therefore, Garissa-township (n = 50), 4 samples (8.0%), were positive while 46 samples (92.0%) were tested negative. from Dhadhab District (n= 50) five (5) samples for (10.0%) tested positive forty-five (45) samples for (90.0%) were negative tested which were higher positive and balambale also had six (6) samples (10.0%) were tested positive. Meanwhile, (54) fifty-four other samples of (90.0%) were tested negative by using competitive enzyme-linked immunosorbent assay (c-ELISA) were considered the strongly positive.

The unit value of competitive enzyme-linked immunosorbent assay (c-ELISA) obtained also indicated the level of antigen from different samples tested according to (Wanjohi *et al.*, 2012).

Double gel diffusion test (DGD-T)

The agar gel immunodiffusion test (AGIDT) test has been used mainly by its high several authors that have reported its special ability to differentiate between S-19 vaccinated and naturally infected animals, when using soluble antigens**.** The test was performed following previous recommendations. Briefly, the gel was prepared in a 10% NaCl, 0.1 of HCl pH 7.2 buffer with 0.7% agarose. Polystyrene 100 mm diameter Petri dishes were filled with 9 ml of the agarose preparation and 3 mm wells punched in a circular pattern were filled with test and control sera, while in a central well the antigen was placed. Readings to detect precipitation lines were done at 24, 48 and 72 hours. A total of one hundred sixty (160) samples of the camel serum from selected slaughterhouses in Garissa sub-counties were tested using the Double Gel Diffusion Test (DGD-T). Eleven (11) samples were tested positive for (6.80%), while one forty-nine 149 negative tested. From Garissa-township District (n = 50), 2 samples for (4.0%), were positive while 48 samples for (28.75%) tested negative. from Dadab District (n = 50) 3 samples for (6.0%) which were positive and Balambale was higher positive with the 6 samples (10.0%) were tested positive and 54 tested negatives. therefore, the bellow figure transforming the different test that used by double gel immuno-diffusion test results from selected slaughtered camel in Garissa County of Kenya.

When sensitivities of the 4 serological tests were compared using by Chi square goodness of fit test, there was no significant difference between them, with respect to picking of positive cases (p was = 0.0999). Then gives the comparative results (percent) for the 4 serological tests, with respect to the study areas. However, the comparison of the four (4) tests, there was no statistically significant difference between the tests.

Table 5: *Statistical significant of a Comparative results (percent) for the 4 serological tests, with respect to the study areas.*

Tests	Township (n=50)	Dadaab (n=50)	Balambale (n=60)	Total No (n=160)
RBPT	4(8%)	6(%)	5(8.3%)	15(9.3%)
SAT	4(8%)	6(12%)	6(10%)	16(10%)
c-ELISA	4(8%)	5(10%)	6(10%)	15(9.3%)
AGID	2(4%)	3(6%)	6(10%)	11(6.8%)
Average	4(8%)	5(10%)	6(10%)	14(8.75%)

Cross-sectional study results

In total of one hundred and sixty (160) camels were inspected to examine for gross pathological lesions and histological lesions. Fifty (31%) were slaughtered at Garissa-township, fifty (31%) at dadaab and sixty (37%) at Balambale slaughterhouses.

Numbers of organ condemned

Of the160 camels that were inspected and examined, 78 (48.75%) of them had at least one pathological condition, 55 (70.5%) had one type of condition and 19(24.4%) had more than pathological lesions, while 38(48.7%) had no organ condemnation. At Garissa-township slaughterhouse, of 50 camels slaughtered 35(70%) had one (1) pathological lesion, (14%) had more than one (1) pathological conditions, while the other 8(16%) had no organ condemnation. At dadaab 50 camel were slaughtered, 30(60%) had one (1) pathological lesions, 10(20%) had same pathological conditions while other 10(20%) had no organ condemnation. At Balambale slaughterhouses, of 60 camels were slaughtered 40(66.6%) had no organ condemnation, 15 (25%) had more than one pathological lesions while others 5 (8.3%) had one same pathological lesions at the slaughter indicated Table 6.

Table 6: *Number of pathological lesions of condemned organs, non-condemned and their %percentage proportion at the three study slaughterhouses.*

Numbers of organs	Slaughterhouses			Total (%)
	Garissa township	Dadaab	Balambale	
Number of 1 pathology	35	18	25	78(48)
Numbers with 1 condition	20	13	22	55(70)
Condemnation numbers	7	6	7	19(24)
No. condemnation numbers	16	8	14	38(48)
Total inspected	50	50	60	160

Organs of condemnation

Among the One hundred and sixty (160) slaughtered camels, 78 (48.7%) were lymph nodes. For Garissa-township48(61.5%),atdadaab18(23%)hadcondemned as whole, and Balambale 12(15.3%) were partially condemned 28(17.5%) of livers were condemned. For Garissa-township slaughterhouses 12(42.8%) had condemned as whole, at dadaab slaughterhouses 9(32.2%) were condemned as partially while Balambale slaughterhouses 7(25%) were condemned as whole 18(11.2%) of lung had also condemned at Garissa-township, 5(27.7%) has condemned as whole, at dadaab slaughterhouses 7(38.8%) had condemned as whole, while At Balambale slaughterhouses 3(16.6%) were condemned partially 20 (12.5%) kidney Garissa-township 9(45%), 6(30%) at dadaab and 5(25%) at Balambale slaughterhouses were condemned as whole 16(10%) heart muscle, 4(25%) at Township, 5(31.2%) at dadaab slaughterhouses and 7(43.7%) at Balambale slaughterhouses were condemned as partially Table 7.

Table 7: *Types of condemned organs and their % percentage proportion at the three different study site slaughterhouses.*

Organs	Township SL	Dadaab SL	Balambale SL	Total
Lymph node	48(61.5%)	18(23%)	12(15.3%)	78
Liver	12(42.8%)	9(32.2%)	7(25%)	28
Lung	6(33.3%)	7(38.8%)	5(27.7%)	18
Heart muscle	4(25%)	5(31.2%)	7(43.7%)	16
Kidney	9(45%)	6(30%)	5(25%)	20
Total of inspected	79	45	36	160

Clinical, gross and histopathology study results

A total of 160 samples were collected and examined during the period of study for laboratory analysis both gross and histopathology. According to the ante-mortem record, clinical manifestation was lameness 48(30.0%), swollen of lymph node 39(24.0%) (Figure 1), Orchitis 6(3.70%), infertility 7(4.3%), Abortion 8(5.0%), Abdominal pain 7(4.3%), decreased milk yield 7(4.3%), inflammation of testicles 6(3.7%), epididymitis 6(3.7%), Anorexia 7(4.3%), in appetence 7(4.3%), infection of urogenital 7(4.3%), and placental infection 6(3.7%). For more clinical manifestation that based slaughtered camel and examined animals.

For the gross condemned lesions encountered had include: fibrin depositions 7(4.3%), enlargement of lung 6(3.7%), pericarditis 38(23.7%), and hepatomegaly with nodular liver lesions 79(49.3%), enteritis 5(3.1%), haemorrhages 6(3.7%), congestion 8(5.0%), of visceral organs (lung and kidney) and abscess of lymph nodes 3(1.8%). the other gross pathological lesions based on slaughterhouses in Garissa County

The histopathology in counted were included cellular infiltrations 15 (6.2%), hypoplasia 3 (1.8%), collapse of alveoli 7 (4.3%), oedema 4 (2.5%), congestions 6 (3.7%), fatty degeneration 5 (3.1%), haemorrhages 9 (5.6%), immunoblastic infiltrations 8 (5.0%), increase in number of lymphocytes 9 (5.6%), pneumonia 10 (6.2%), lymphoblastic infiltrations 8 (5.0%),

Table 8: *Pathological changes of condemned organs with the respect to Brucella sero-reactants in slaughtered camels in Garissa County.*

Camel No.	Cond.organs	Clini. Signs	Gross Lesions	Histopathology
SC-GT-12	Lymph node	Swollen	Enlargement and abscess	Cellular infiltration
	Stomach	loss of appetite	Discoloration	Haemorrhages
SC-GT-14	Lung	Lameness	Change in colure , white spots	collapse of alveoli, pinkish fluid materials with the alveoli (Oedema)
SC-GT-24	Liver	Lameness	Distended	Fatty degeneration
	Liver	Placental infection	Thickened of bile duct	Diffuse of fatty infiltrations
SC-GT-29	Lymph node	Swollen of lymph nodes	Swollen	immunoblastic infiltration
SC-GT-30	Heart	Anorexia	fibrins and haemorrhages	destructions of fibrins
SC-DA-64	Lung	In appetence	Discoloration	Pneumonia
SC-DA-69	Heart	Placental infection	Congested	Lymphoblastic infiltrations
SC-DA-70	Kidney	Epididymitis	Congested	Congestion and haemorrhages
SC-BA-120	Heart	Infertility	Fibrins	Slightly destruction of fibrins
SC-BA-132	Lung	Abdominal pain	Congested	Polymorph-nuclei in Alveoli
SC-BA-143	Kidney	Abortion		
SC-BA-144	Lymph node	Swollen of lymph nodes	Enlarged in some areas	Increase number of lymphocytes
SC-BA-150	Heart	Infection of urogenital	Haemorrhages	Macrophages and neutrophil infiltrations
SC-BA-155	Liver	Anoxia	Hepatomegaly	Oedematous mononuclear cells
SC-BA-158	Kidney	Abortion	Congested	Congested and haemorrhages

fibrosis 7 (4.3%), macrophages and neutrophils 10 (6.2%), inflammatory cells 9 (5.6%), cellular injuries 8 (5.0%), accumulated of blood cells 8 (5.0%), compensatory Emphysema 9 (5.6%), increase in number of hepatocyte cells 10 (6.2%), inflammatory lesions in skin 8 (5.0%), and also recorded necrosis in myocardium 7 (4.3%). Therefore, the pathological changes of sero-reactants in condemned organs Table 8, with the respect of *Brucellosis* in slaughtered camel in Garissa County. For more pathological changes.

Gross morphology and histopathology appearances for the positive condemned organs lymph node

The examination of lymph node tissue from slaughtered camel in Garissa-Township whose sera was tested positive to the brucellosis had immunoblastic infiltrations, hypoplasia (meaning that underdevelopment or incomplete development of a tissue or organ), and few mature lymphocytes and increase number of lymphocytic cells were observed in lymph nodes for grossly and histopathology features. The lymph node of sampled from negative camel ten (10) and twelve (12) had similar lesions.

Lung tissue

The lung tissue from positive camel with brucellosis had verified collapse of alveoli, pinkish fluid materials with the alveoli (Oedema), Pneumonia, infiltrations of polymorphonuclear cells (is a type of immune cell that has granules (small particles) with enzymes that are released during infections, allergic reactions, and also asthma) and neutrophils in bronchioles for grossly and histopathology photomicrography However, the lung had thickened alveolar walls with interstitial mononuclear infiltrate, congestion in some areas and haemorrhages. There was negative sampled lung tissue of camel five and nine showed similar lesions.

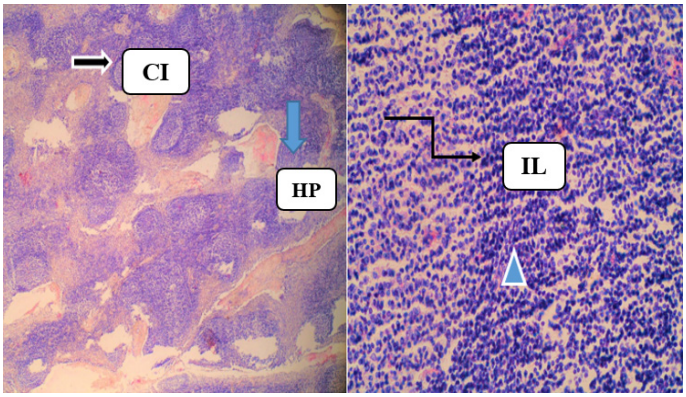

Figure 1: *Histopathology of Lymph node for brucellosis-positive Camel case number (SC-12) and were viewed that the immunoblastic infiltration, Cellular infiltration (CI); hypoplasia of lymphocytes (HP) and increase number of lymphocytes (IL) ((H/E × 40x and 400x).*

Heart muscle

The heart tissue from seropositive camel of brucellosis had heavy fatty degeneration, lymphoblastic

infiltrations in cardiac muscles, slightly destructions of fibrins in cardiac, in some areas there was macrophages and neutrophilic infiltrations for grossly and histopathology photomicrography and also inflammatory cells are more in heart section. However, in a sampled sero-negatives camels eight (8) and Nine (9) had similar pathological lesions.

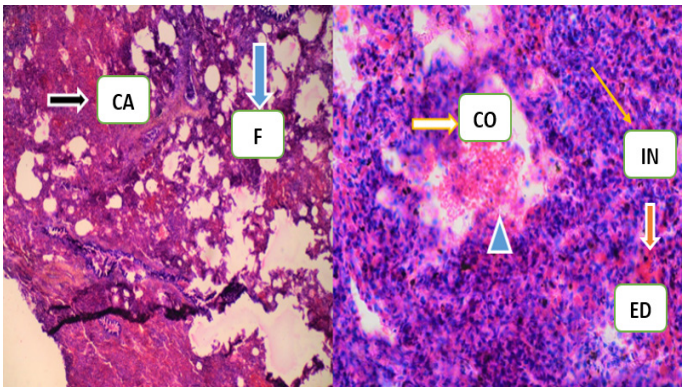

Figure 2: *The histopathology feature of lung tissue for positive –brucellosis obtained from camel case number SC-GT-14 were publicized that collapse of alveoli (CA), pinkish fluid materials (F) in alveoli (Oedema) (ED), Pneumonia, infiltrations of polymorph-nuclear cells (IN) and heavy congestion (CO) (H/E × 40x and 400x).*

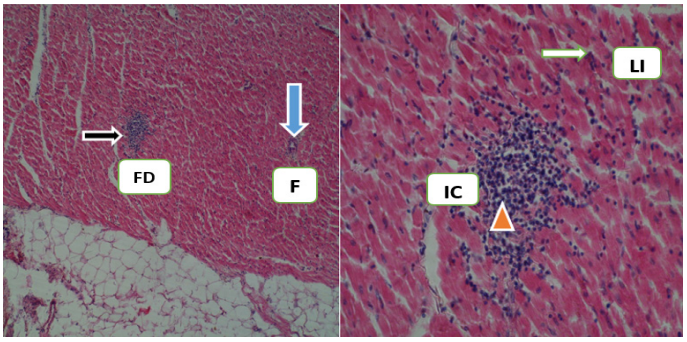

Figure 3: *Those two (2) features were mad by Histological features of heart organ from zero –Positive tested camel obtained from Sample camel number (SC-GT- 30) were revealed that fatty degeneration (FD), lymphoblastic infiltration (LI) in cardiac muscles, slightly destructions of fibrous (F), macrophages, neutrophilic infiltrations in some areas and inflammatory cells (IC) (H/E × 40x and 100x).*

Liver

The liver tissue from seropositive camel of brucellosis was viewed fatty degeneration, Neutrophils, liver injuries, diffuse fatty infiltration (meaning that accumulation of excess fat in the liver) mononuclear cells, lymphoblastic cellular of infiltration and congestions in some areas for gross morphology of condemnation and features of histopathology photomicrography slight blockage of vessels and macrophages in live and enlargement of hepatocytes. Sampled liver with sero-negatives camel six and seven had similar pathological lesions.

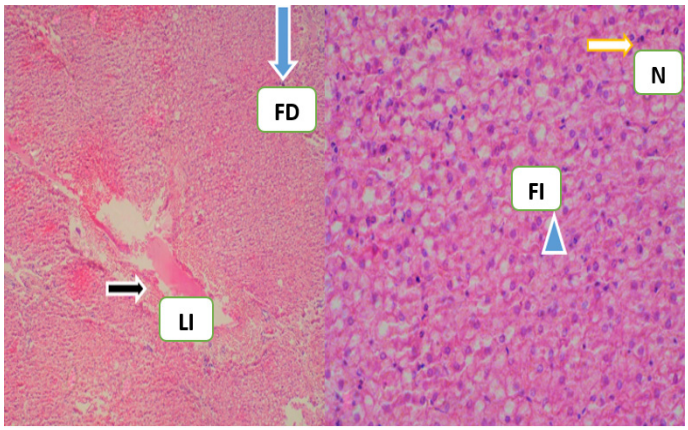

Figure 4: *Histological section of condemned liver with tested seropositive obtained from sample camel Number (SC-24) was specified fatty degeneration (FD), Neutrophils (N), liver injuries (LI), diffuse fatty infiltration (FI) (H/E × 40x and 100x).*

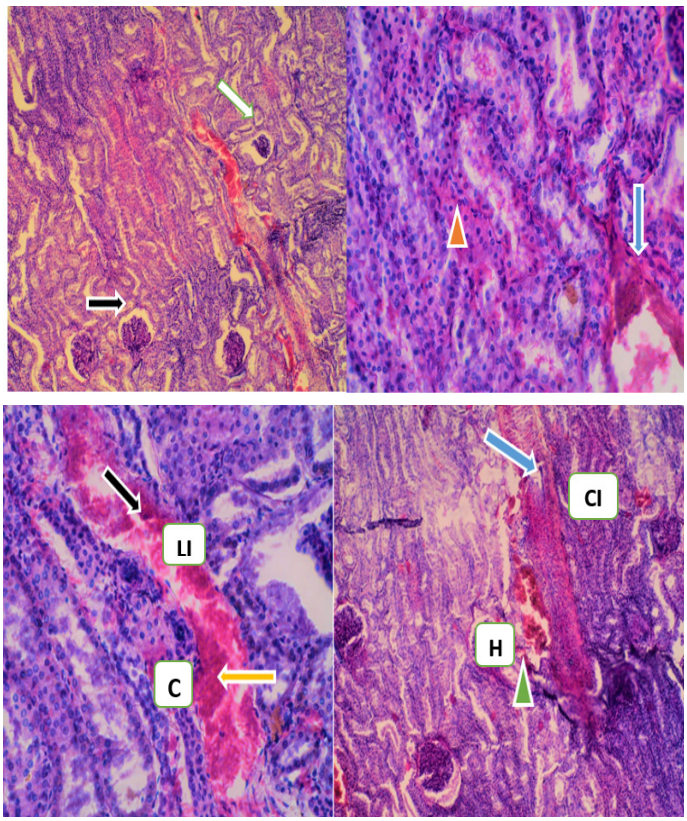

Figure 5: *The histlogical features of kidney with tested seropositive obtained from sample camel numbe (SC-70) was pointed infiltration lymphoblastic cells (LI),congestions (C),cellulr infiltaraions (CI) and hemorhages (H) (H/E ×40, 400x and 100x).*

Kidney

The kidney tissue from seroposive camel brucellosis were shown diifernt pathological lesions lymphoblastic cells of infiltration,congestions and hemorhages in some arreas for gross and histopathology photomicrography and also there was inflamatory cells with the perodominantly hetrophils in kidneys. The other kidney sampled of seronegetive from number thirteen and seven had simillar pathological leisions.

Results and Discussion

This study in Garissa camel slaughterhouses providing that a valuable occasion for producing with the camels' prevalence data and pathological lesions that could be related with human health awareness. To determine the sero-prevalence of camel brucellosis, Four (4) different serological survey was conducted in camel slaughterhouses including the examination of blood specimens from different camel slaughterhouses in Garissa County and used, Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Competitive Enzyme Linked Immune Sorbent Assay (c-ELISA) and Double Ager Gel Immunodiffusion Test (DAGID-T).

Sero-prevalence estimated that around 10% of 160/15 tested samples similar to (Alhaji *et al*., 2016). Understanding to the pathology and occurrences of *Brucellosis* in camel are important in veterinary problems that relating to the production losses and abortion. Therefore, the zoonotic diseases means that are also significant for medical professions to know about the in livestock. To establish the pathological lesions of *apparent-Brucellosis* in slaughtered camel, followed by the examination of sero-positive and serf-negative tested in serology for grossly and histologically.

Prospective study: Sero-prevalence

A total of one hundred and sixty (160) camel serum samples from Garissa slaughterhouses were confirmed by using Rose Bangle Plate Test (RBPT). Fifteen (15) samples (10%) tested positive. From Garissa-township (n=50) four (4) samples (8.0%) were tested positive, fifty (n=50) samples from dadaab slaughterhouses six (6) (12.0%) were tested positive while sixty (n=60) samples from Balambale slaughterhouses five (5) (8.3%) were tested positive. The one hundred and sixty (160) camel serum samples from Garissa County slaughterhouse were also tested by using serum agglutination test (SAT). Sixteen (16) samples (10.50%) were tested positive. From Garissa-township four (4) samples (8.0%) were tested positive. From dadaab six (6) samples (12.0%) tested positive. From Balambale six (6) samples (10.0%) were tested positive. For the 16 positive samples 10 samples had a titre of 1/10, 3 samples a titre of 1/20, 2 samples attire of 1/40, and 2 sample had a titre of 1/80 and 3 samples had a titre of 1/160. Similarly reported prevalence (Wanjohi *et al*., 2012).

The one hundred and sixty (160) had also tested using by Competitive Enzyme Linked Immune Sorbent Assay (c-ELISA). Fifteen (15) samples (9.3%) were tested positive. From Garissa-Township central of Garissa County (n=50), four (4) samples were tested positive. From dadaab (n=50), five (5) samples had tested positive. For Balambale (n=60), six (6) samples were tested positive. As reported similarly (Njeru, *et al*. 2016). The one hundred and sixty (160) camel serum samples from Garissa slaughterhouses were also tested by using AGID Test. eleven (11) samples (6.8%) were tested positive. From Garissa-Township (n=50), two (2) sera samples (4.0%) were tested positive. From dadaab (n=50), three (3) sera samples (6.0%), were tested positive. And from Balambale (n=60), six (6) sera samples had tested positive by using chi-square ($\chi 2$), there was no statistical difference in sensitivity between the four serological tests (p=0.999).

The present study for sero-prevalence result findings (10%) is similar to the previous reports from the different countries (Wanjohi *et al*., 2012). However, there was lower than some studies in Somalia, Tanzania, Ethiopia, Nigeria, Saudi Arabia and Yemen. Ser-prevalence was differed from other findings in neighbouring countries of Kenya (in the Afar region of Northeast Ethiopia. Individually, in the lower sero-prevalence in this study is not consistence with the other prevalence findings which showed that the infection is more prevalence among nomadic slaughterhouses in Garissa County Kenya. The sero-prevalence of *Brucellosis* in camel was lower in extensively kept pastoralists of camel in Garissa-township and dadaab slaughterhouses, while on the other hand had been reported in intensively kept pastoralists of camel was higher in Balambale slaughterhouses. Thus, several factors may affect increasing result of serological outcomes such as production system, overcrowding of restricted area, contacts between the animals, immune suppressive effective of trypanosomiasis that often prevalence in camel and cross-reacting bacteria of *E-coli, Salmonella* and *Yersinia* and uses of lower specificity tests. These factors have potential effects for serological findings. The sample sections and sampling for different animals may also be affect higher prevalence for the serology study. The higher prevalence of brucellosis represents the major challenges of both economics and public health problems. It is prospective that there is higher frequency of abortion/reproductive failures that may lead to the potential higher level of

exposures of livestock owners and their families. It was very important to know that the RBPT is good diagnostic sensitivity compared to the other there (3) serological testes that have been done to the survey. So that, the RBPT is satisfactory screening test the test procedure for diagnosis of bovine brucellosis to be applied for camel brucellosis. Though camels are not known to be the host of *Brucella* organism, but it is well known to be susceptible for *Brucella abortus* and *Brucella melitensis*. Therefore, the disease is still remaining wildlife and domestic animals from the sources of human infection through, direct contacts and contamination of environment during parturition and abortion. Although, the infection in camel has been reported in Saudi Arabia, Sudan, Kenya, and Tanzania, Ethiopia and Somalia and other countries in the world. Generally, to control of the disease both animals and manes we need to keep the following: (1) improvising the hygiene (to reduce the direct contacts between infected and non-infected animals), (2) public awareness (to control and prevent the infection) and (3) proper disposal (to be disposed the effected foetus, tissues, discharges and poste-mortem equipment and to infect the contemned utensils).

Therefore, this study has been confirmed the presence of brucellosis in Garissa slaughterhouses of Kenya showing that the significant of sero-prevalence of (10% tested with RBPT, SAT c-ELISA and AGID). Further studies are more needed to improve the production of camel and diminish the risk transmissions of the infection to the human especially benchers. There is also needed control program for brucellosis in camel slaughterhouses and other animals. Standard biosecurity measures at slaughterhouses and farms be enhanced to control and prevent of *Brucella* infection to animals and human.

Cross-sectional study: Pathological lesions

The cross-sectional study 48% of slaughtered camel had one or more contaminations of organ at Garissa-township, dadab and Balambale slaughterhouses. Up on the histological features the main cause of contamination apart from *Brucellosis* were: Circulatory disturbance, inflammatory conditions.

The clinical manifestation of the slaughtered camel embraced swollen of lymph nodes (24%), sever lameness (30%) and abortion (5%). In the swollen of lymph nodes of the effected and non-effected of *Brucellosis* in slaughtered camels were enlarged and abscess that attributed to obstruction and discolorations of fluid. Microscopically, due to the miss stained of the slide some area appeared disorganized, cellular infiltrations, mononuclear inflammatory cells, immunoblastic infiltrations, increase number of lymphocytes and hypoplasia meaning that (underdevelopment or incomplete development of a tissue or organ). These lesions there was similar study in camel lymph nodes that come across in Sudan (Aljameel *et al*., 2013) and in Yemen (Hamza *et al*., 2017).

There is also liver of three (1.8%) obtained from the positive tested *Brucellosis* which had clinical manifestation of lameness at the anti-mortem record. Histopathologically, Fatty degeneration, Diffuse of fatty infiltrations, fibrosis, hepatocyte denegation and in some area necrosis. Similarly, to in Iran (Khaniki *et al*., 2013) and in Saudi Arabia (Mohamed, 2013). The adjusted area of the liver there was injury, congestion convoyed to inflammatory infiltrated cells and hepatocyte degenerations in some area. These grossly and histopathology findings generally decides with the study of Khaniki *et al*. (2013).

The lung of two (1.2%) from positive slaughtered camel was rejected at the slaughterhouses in dadab due to the enlargement, discoloration, white and red spots and fluid filed with cyst from the surface. Microscopically, collapse of alveoli, pinkish fluid materials with the alveoli (Oedema), mononuclear infiltrations of cells, slight blockage of vessels and macrophages, enlargement of hepatocyte cells. The adjacent area of bronchioles were congested a accompanied by slightly inflammatory cells. Similarly, and also, there a study in Saudi Arabia (Gameel and Yassein, 2010).

A heart from 4(2.5%) of tested positive *Brucellosis* that rejected to supply the slaughterhouses were condemned fibrins and haemorrhages. These gross conditions were also met at the slaughterhouse. Histopathological examinations, fibrins and haemorrhages, destructions of fibrins, lymphoblastic infiltrations, Macrophages and neutrophil infiltrations, fatty degenerations and inflammatory cells were found. Similar study has been done in Tanzania (Tembo and Neonga, 2015) and in Bangladesh (Mazumder *et al*., 2012).

Kidney of 2 (1.2%) from camel slaughtered were also condemned during the poste-mortem inspection as they were discoloured and congested and

haemorrhages with white-dark-red under the renal cortex microscopically, conformed that the presence of inflammation in cells, infiltration, macrophages, haemorrhages and congestion. Previous studies had also done from lung tissue of slaughtered camel in Athi River, Kenya (Mutua *et al*., 2017).

The lung from 2(1.2%) slaughtered camel obtained from sero-negative sample that were also condemned during the poste-mortem inspection with the red-dark coloured under pleural cavity. These conditions were also come across at the slaughter. Histological examination, confirmed that presence of erythrocytes and pinkish materials in bronchi and bronchioles and there were symptoms indicating inflammation in the slaughtered camel lung. Similar study has done in Ethiopia with the absence of inflammatory cells in lung (Mamo *et al*., 2011).

Finally, there is correlation between the gross pathology and microscopically examination.

Conclusions and Recommendations

The sero-positivty demonstrated by the camel brucellosis brought-in for slaughter was about 10% this indicates that the disease is enzootic in the area, though the figure is lower than what has been reported in other areas of the county. The infection has both economic and public health importance; it is zoonotic.

- Camels from Balambale slaughterhouses showed higher sero-positivity, using by the four (4) different serological test.
- Camels that were sero-positive also had clinical and pathological lesions similar to the those brucellosis.
- The despite of the prevalence of the disease been below referred to other results to the county, but the disease is still posing a public health problem economic loss to the slaughterhouses in the county because of its zoonotic nature and its clinical manifestations that being similar to other disease.
- There was correlation of the positive tested animal, clinical and pathological lesion that observed in the different test of the study.
- There were some health canters in the county that carry out limits test of brucellosis in camel slaughterhouses but faces the shortage of test reagents and to record is inconsistence and unpredictable to this work.
- There is lack of awareness on risk factors of the infection into the slaughterhouse's owners because of the methods of prevention and control strategies. Therefore, the risk factors are significant in the spread of the disease.
- There was a number of condemned organs due to the infectious and non-infectious that contributed by the sanitation levels of poor slaughtering of the animals such liver, lung and other visceral organs which is good for human consumption. And it was also taking parts to the economic losses of slaughterhouses in the county.

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Author's Contribution

Manually they have contribute all authors and some of them we have used Google scholar

Conflict of interest

The authors have declared no conflict of interest.

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