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SERO-Prevalence of Brucellosis in Camel Slaughter-Population in Garissa County, Kenya

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Abstract
Camel brucellosis is an infectious disease, mostly presenting in chronic state; caused by Brucella organisms, which also affect other animals. Species that have been documented to affect camels are: Brucella abortus (normally causing disease in cattle) and Brucella mellitensis (normally causing disease in goats). The disease has been shown to spread between animals, across herds and to humans. This study was aimed at establishing sero-prevalence of brucellosis in Garissa camels. It was a cross-sectional study, where the study population was purposively chosen to consist animals taken for slaughter in 13 slaughterhouses within three sub-counties of Garissa County, namely Garissa Central (represented by Garissa-township), Garissa East (represented by Dadaab) and Garissa West (represented by Balambale) . This was because the slaughterhouses normally handle many camels in a day, thus making it easy for the investigator to access the required number conveniently; it was also assumed that data collected from these for-slaughter camels was representative of the situation in the subcounty/county. A total of one hundred and sixty camels were tested using four serological tests: Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Competitive- Enzyme Linked Immuno Sorbent Assay (c-ELISA) and Double Agar Gel Immunodiffusion Test (AGID). The serological tests were purposively chosen to increase the chances of picking positive cases and also to compare their sensitivities, with respect to camel serum, since they were originally meant for use on bovine serum. Blood samples (15 ml) were collected for serum harvesting from jugular veins of the animals as they were waiting to be slaughtered. Rose Bengal plate test and SAT were run at a laboratory within the slaughterhouse premises; cELISA and DAGIT were run at department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, Kabete campus; serum samples having been transported in a cool box. On average, out of an overall total of 160 serum samples tested, 14 gave positive results, amounting to a prevalence of 8.75%. For the three counties, respective prevalence (averaged from the 4 serological tests run) were: 8% (4/50) for Garissa -township; 10% (5/50) for Dadaab and 10% (6/60) for Balambale. When sensitivities of the 4 serological tests were compared, there was no significant difference between them, with respect to picking of positive cases (p=0.05), The study has demonstrated presence of brucellosis in camels in Garissa County and the authors are, recommending usage of RBPT as a screening test, since it is cheap, quick, and easy to carry-out. Any of the other three more involving tests can then be used if one wants to establish respective titres.

Keywords: *Camel; Brucellosis; Slaughterhouse; Garissa, Kenya.*

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I. Introduction

Camel is a multipurpose animal and has long been domesticated by humans. People of North Eastern region, including Garissa, keep camels for economic and social purposes, such as for milk, meat and hide supply, and for other purposes like transport, entertainment, celebration and competition as in racing and beauty show (Kaskous *et al.* 2016).

Camel is the dominant livestock in the area, where it provides sustenance to several individuals, particularly since the area is prone to frequent droughts, which kill other animals (sheep, goat and cattle) or make them unthrifty (Wanjohi, *et.al* 2012). About 60% of Garissa County populace are pastoralists and keep around 300,000 camels; while

FAO report (2009), puts the Kenyan camel population at about 600,000. Camels are also important globally, for both industrial and financial reasons (FAO 2009).

There are three types of camel kept in the North-Eastern region, namely: Turkana type (which is small-sized; average weight 350 kg); Rendille/Gabbra type (average weight 300 kg) and Somali type (which is the biggest; average body weight 550 kg) (FAO 2009).

Camel brucellosis has been reported in all camel-raising nations (Magwisha, *et al.* (2016). It is an infectious disease; mostly presenting in chronic state; caused by *Brucella* organisms, which also affect other

animals. Species that have been documented to affect camels are: *Brucella abortus* (normally causing disease in cattle) and *Brucella mellitensis* (normally causing disease in goats) (Perrett *et al.*2008). The disease has been shown to spread between animals, across herds to humans and across countries (Garcell *et.al.* (2016); it is, therefore, an important zoonotic disease. In terms of camel production, brucellosis prevalence is higher in intensive camel rearing farms than in those practising extensive farming system (Abbas and Agab 2002). Transmission is always from infected animal(s); humans getting infected through drinking of uncooked milk, meats (e.g. liver and kidney) from infected animal; and through close contact with infected animal(s) (e.g. when slaughtering) by inhalation of contaminated air or dust (Musallam, *et.al*, 2016; Moreno 2014).

Brucella organisms affect various organs/tissues of the host, most-commonly the uterus, testicles, lymph nodes (Aljameel *et al*. 2013). Organ(s) (visceral or other) have been condemned in camel slaughterhouses as a result of the disease, around the world (Tenaw, *et al.*2015).

There is scarce information on camel brucellosis in Kenya. The only documented study is that of Wanjohi et.al (2012); they reported a sero-prevalence of 15.22% (35/230) in Garissa and Wajir Counties, using Milk Ring Test (MRT), Rose Bengal Plate Test (RBPT) and Serum Agglutination Test. However, there are many unpublished reports of suspect brucellosis cases in camels and humans in Garissa County, especially from slaughterhouses, where diagnosis was based on gross pathological lesions. This study aimed at establishing brucellosis prevalence of camels that were taken for slaughter, in Garissa County. Four serological tests were used, including: Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Competitive- Enzyme Linked Immuno Sorbent Assay (c-ELISA) and Double Agar Gel Immunodiffusion Test (AGIT). The serological tests were purposively chosen to increase the chances of picking positive cases and also to compare their sensitivities, with respect to camel serum, since they were originally meant for use on bovine serum.

II. Materials And Methods

STUDY AREA

The study was carried out in Garissa County (figure 3.1). The county is one of the three Counties in the North Eastern region of Kenya. It is located in Eastern Kenya bordering Somalia to the East, Wajir County and Isiolo County to the North, Tana River County to the West and Lamu County to the South (KNBS, 2015). It lies between latitude 10 58'North and 20 1' South and longitude 380 34' E and 410 32' E.and It covers an area of 44,174.1 Km^2 and is arranged at 0.46° South scope, 39.66° East longitude and 152 meters height over the ocean level.(GOK 20114).Agriculture and livestock are pilar of the county economy. They are maine sources of occupation and livelihood for farmers and others in the value chaine.

In the county there are twenty (20) camel slaughter facilaties. Six (6) are located in blambale sub-county. three (3) is in Dadaab,four (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).

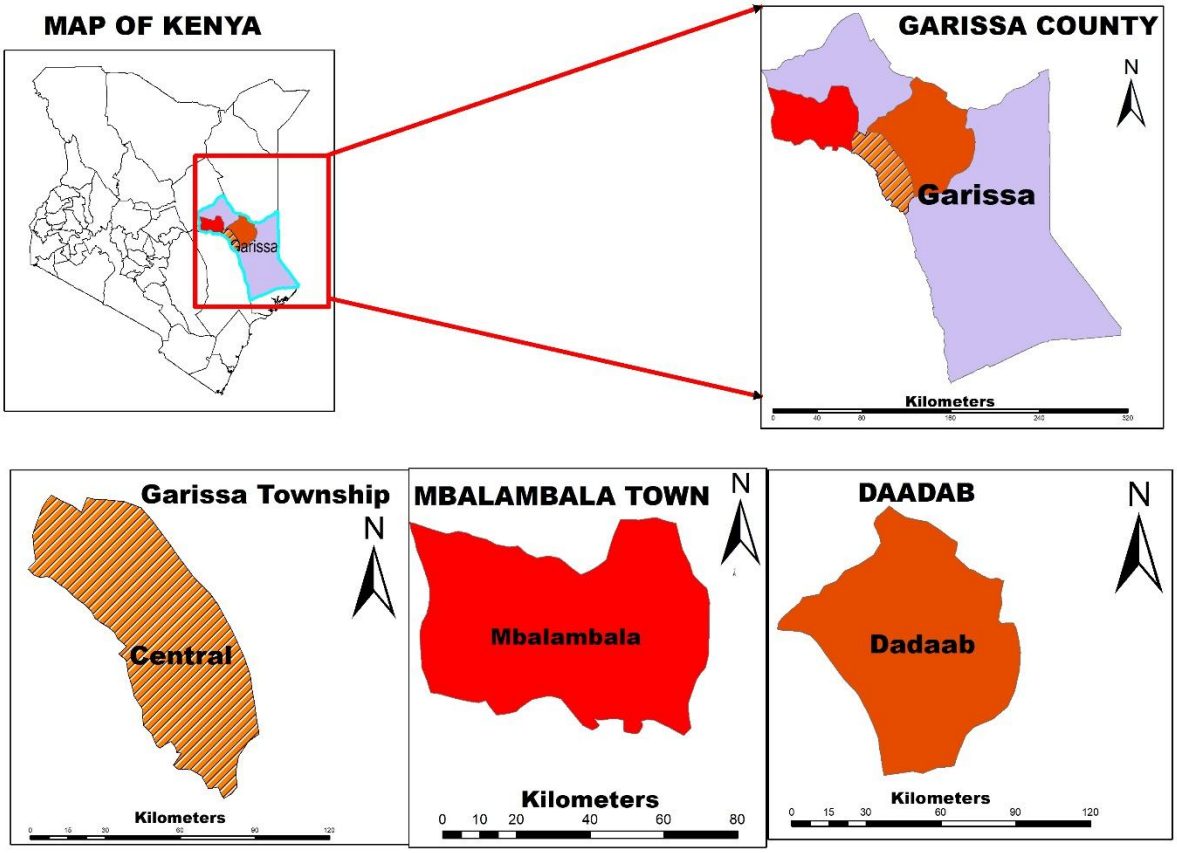

Figure:1 Study Area

STUDY DESIGN

This study aimed at establishing *Brucella* sero-prevalence in Garissa camels; it was done between September 2018 and March 2019. A total of one hundred and sixty camels were tested using four serological tests: Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Competitive- Enzyme Linked Immuno Sorbent Assay(c-ELISA) and Double Agar Gel Immunodiffusion Test (AGID).Rose Bengal plate test and SAT were run at a laboratory within the slaughterhouse premises; c-ELISA and AGID were run at department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, Kabete campus; serum samples having been transported in a cool box. The results were then compared statistically using Chi square goodness of fit test.

SAMPLING DESIGN

The study camel population was purposively chosen to consist animals taken for slaughter in three sub-counties of Garissa County, namely Garissa Central (represented by Garissa-township), Garissa East (represented by Dadaab) and Garissa west (represented by Balambale). This was because the slaughterhouses normally handle many camels in a day, thus making it convenient for the investigator to access the required number; it was also assumed that data collected from these for-slaughter camels was representative of the situation in the sub county/county. However, the individual slaughterhouses for each sub-county were selected randomly. It was also on purpose that 4 serological tests were used in this study; reason being to increase the chances of picking positive cases and also to be able to compare sensitivity of the tests, with respect to camel serum, since they were originally meant for use on bovine serum.

Blood sample collection and harvesting of serum

Blood samples (15 ml) for serum harvesting were collected from jugular veins of the animals as they were waiting to be slaughtered. The blood was put into sterile bottles, which were kept at slanting position in a cool box as they were transported to the laboratory at the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi. At the laboratory, they were kept at room temperature for a further 2 hours, after which they were centrifuged at 4,000-4,500 × gram for 15 minutes and serum harvested into fresh sterile bottles, labelled and stored at -20o C until when tested using the 4 tests. Before testing, the samples were kept at room temperature for 2 hours, in order to thaw and warm-up to the room temperature level.

Rose Bengal Plate test (RBT)

The Rose Bengal test (RBT) was carried out using the method of Ducrotoy et al. (2016) and OIE (2016); 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^{o}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.

Serum Agglutination Test (SAT)

This test was carried out using the method of OIE (2016); the 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.

Enzyme Linked Immuno-sorbent Assay (c-ELISA) Tests

The competitive Enzyme Linked Immunosorbent Assay(c-ELISA) ID screen: COPMELISA 400 (RAI 2009)-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 test used (Mariam *et.al, (*2016).

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 3.1: 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* anti-body detection was used; following the method of (Waggett *et al.* 2013) 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 line(s) showed positive reaction. Positive and negative control ware also set-up.

III. Results

One hundred and sixty (160) camel serum samples from Garissa sub-counties selected slaughterhouses were tested Using four (4) serological tests: Rose Bangle Plate test (RBPT) Serum Agglutination test (SAT), Competitive Enzyme Immuno-Sorbent Assay test (c-ELISA) and Agar Gel Immuno-diffusion test (AGID). For the RBPT, 15 samples were tested positive (Table 1). 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 1: 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)

For SAT, 16 out of the 160 samples (10.0%) tested positive (Table 2): 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. Dadaab had the highest percent positive reactor rate of 12.0% (6/50); Balambale had reactor rate of 10.0% (6/60); Garissa-township had reactor rate of 8.0% (4/50).

Table 2: 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

Competitive Enzyme Linked Immunosorbent Assay Test (cELISA)

For cELISA, 15 out of the 160 samples (9.3%) tested positive (Table 3): 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. Dhadhaab had the highest percent positive reactor rate of 12.0% ((6/50); Balambale had reactor rate of 8.3% (5/60); Garissa township had reactor rate of 8.0% (4/50).

Table 4.3: 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

Ager Gel Immuno-diffusion Test (DGD-T)

For Agar Gel Diffusion test (AGID), 11 out of the 160 serum samples (6.8%) gave positive results (Table 4); 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. Balambale had highest percent reactor rate of 10.0 %(6/60); Dhadhab had reactor rate of 6.0% (3/50); Garissa-township had reactor rate of 4.0% (2/ 50).

Iindividuals /Groups	***B. melitensis***	***B. abourtus***	**Percentage (%)**
Infected camel of Garissa-township	Se (%)	Sp (%)	
(n=2)	1	1	**2.0**
infected camel for Dhadhaab	Se (%)	Sp (%)	
(n=3)	2	4	**6.0**
Infected camel for Balambale	Se (%)	Sp (%)	**10.0**
(n=6)	4	6	

Total number of infected camels (n=11)	Se (%) 3.43	Sp (%) 3.43	**6.875**
Total number of non-infected camel (n=149)	Se (%) 46.52	Sp (%) 46.52	**93.12**
Vaccinated slaughtered Camel (n=61)	Se (%) 19.06	Sp (%) 19.06	**38.12**

Table 4: Gel Diffusion Test (AGID) results overall and with respect to the three study areas of Garissa County, Kenya

Se = sensitivity;

Sp = specificity;

DVSC = Different vaccinated Slaughtered Camel.

When sensitivities of the 4 serological tests were compared, using the Chi square goodness of fit test, there was no significant difference between them, with respect to picking of positive cases (p was = 0.0999**).** Figure 1 gives the comparative results (percent) for the 4 serological tests, with respect to the study areas.

Figure1**: 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%)

Therefore, there was no statistically different for the four (4) tests

IV. Discussion

This study in Garissa camel slaughterhouses provided a valuable opportunity for generating with the camels' prevalence data that could be linked 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 (AGID).

Sero-prevalence estimated that around 10% of 160/15 tested samples similar to (Alhaji *et al.* 2016). A total of one hundred and sixty (160) camel serum samples from Garissa slaughterhouses were tested 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. Similarly (Gwida, *et.al.* 2011). 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. Similarly to (Obonyo& Gufu 2015).

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 (Junaidu *et.al*, (2006); Dawood (2008); wanjohi *et.al* (2012). However, there was lower than some studies in Somalia (Abbas & Agab (2002), Somaliland Ghanem *et al.* (2009), Tanzania (Assenga, *et al.* (2015), Ethiopia (Teshome *et al.* (2003), Nigeria. (Junaidu *et al.* (2006); Madu, *et al.* (2016), Saudi Arabia (Radwan *et al* (1992), and Yemen (Al-Garadi, *et al.* (2015). Prevalence was differed from other findings in neighbouring countries of Kenya (in the Afar region of Northeast Ethiopia (Hadush, *et.al*, (2013). Individually, in the lower prevalence in the study is not consistence with the other findings which showed that the disease is more prevalence among nomadic slaughterhouses in Garissa county Kenya. The 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 effect higher prevalence for the serology study. The higher prevalence of brucellosis represents the major challenges of both economics and public health problems. It is likely that there is higher incidence 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 (Gessese *et al.* (2014). So that, the RBPT is satisfactory screening test as (OIE: recommended in 2012) the test procedure for diagnosis of bovine brucellosis to be applied for camel brucellosis. Though camels are not know 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 fetus, tissues, discharges and poste-mortem equipment and to infect the contamined 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.

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