

## Prevalence And Knowledge Of Brucellosis In Dairy Cattle In Makuyu Division, Murang'a County, Kenya

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### ABSTRACT

Brucellosis is a cosmopolitan zoonotic disease that affects man, domestic animals and wildlife. The bacteria *Brucella abortus* is the principle cause of brucellosis in cattle. The incidence of the disease in humans, and which directly relates to that in other animals, is highly dependent on animal husbandry practices, the interaction between humans and animals, living standards, hygiene, food customs, and animal and human population density. Makuyu Division where the study was undertaken experiences hot and dry climate, the area has no permanent rivers and domestic water is obtained from boreholes. Most families live in congested homes and are provided with relieve food by the Government. The standard of hygiene is very low and most of the people are poor. In August 2009 six cases of retained placenta disease were reported to Makuyu veterinary Department among other diseases. Most people are readily exposed to infected animals or indirectly to milk, water and environment contaminated by brucellosis. It is for these reasons that the study was undertaken with the aim to determine prevalence of *Brucella abortus* in dairy cattle and assess knowledge of the disease among the dairy farmers. The study involved random sampling of dairy cattle in the division and a representative sample of two hundred and eight milk and blood samples were collected. The samples were screened at the Central Veterinary Laboratories, Kabete. Immunological tests were used to test presence of *Brucella abortus* antibodies. A questionnaire was used to assess knowledge of brucellosis among the dairy farmers and determine utilization and accessibility to veterinary services. Data analysis involved descriptive statistics to obtain prevalence rate. The percentage prevalence of brucellosis in dairy cattle as determined by both MRT and RBPT were 7.7% and 1.9% respectively. Knowledge of brucellosis was significantly related to the age and level of education of the farmers. The young and educated farmers were more knowledgeable on brucellosis than those who were old and not educated. The old and not educated were the majority and they had no idea on what causes brucellosis in cattle. The Ministries of Health and Agriculture, Livestock and Fisheries will use the results in targeting education and prevention programs to address the disease. Farmers will be educated on methods of controlling brucellosis to reduce prevalence, use of artificial insemination to reduce venereal transmission of brucellosis during mating, herd health campaign with frequent screening for brucellosis to identify infected animals so that they can be slaughtered to eradicate the disease and food and occupational hygiene to prevent transmission of brucellosis to human beings.

## INTRODUCTION

### Background information

Brucellosis is often called “undulant fever” or “Bang’s disease,” (Cloeckert *et al.*, 2003). The importance of this disease in humans dates back to 1887 when David Bruce first isolated the causative agent from livers of British soldiers dying in Malta (Young, 2009). In human the clinical signs are mild fever, enlarged lymph nodes, and weight loss. In cattle the clinical signs are abortion, arthritic joints and retained afterbirth (Dricot *et al.*, 2004). Four varieties of the genus *Brucella* cause brucellosis in human. *Brucella abortus* invades cattle; *Brucella canis*, dogs; *Brucella melitensis*, goats and *Brucella suis*, pigs (Gumber *et al.*, 2004). In human *Brucellae* enter the body mainly through ingestion of unpasteurized milk (Hasanjani, 2006). In cattle, transmission occurs by ingestion of organisms in aborted fetuses, foetal membranes, vaginal discharges, contaminated feeds and water (Hong *et al.*, 2000).

Brucellosis is typically a chronic infection of domestic animals involving the mammary glands and the uterus, thereby contaminating milk and causing abortions in the affected animals (Jennings *et al.*, 2007). The most important control measures against brucellosis are pasteurization of dairy products and inspection of domestic animals for evidence of the disease. An attenuated vaccine effectively controls the disease in domestic animals (Seismenis, 1998). In Kenya, prevalence of brucellosis is high in Narok and other pastoral areas where it is endemic. Many cases have been reported in the various annual reports of the ministry of agriculture and other literature (Muriuki *et al.*, 1994). Serological tests for 10 years (1978-1987) at Veterinary

Laboratories in Kabete, revealed that 17.08% cattle (N=16,273) 4% goats (N=2,851) and 7% sheep (N=1,374) were infected (Kagunya and Waiyaki, 1978).

In August 2009, six cases of retained placenta disease were reported to Makuyu veterinary Department among other diseases (DVO, 2009). The Makuyu Health Centre officers reportedly refer about 5% of patients every two months to bigger hospitals on suspicion that they suffer from brucellosis (Dr Kamau-personal communication). It is for this reason that this study was undertaken with the aim of determining prevalence of brucellosis by establishing the reactor rate of randomly sampled dairy cattle in Makuyu Division. It is important to carry out the study so that the Ministries of Public Health and Livestock can use the results in targeting education and prevention programs to address the disease. The farmers can also be educated on how to handle infected animals to prevent transmission of brucellosis to human and the disease control measures in general.

### **General objective**

To determine prevalence and knowledge level of brucellosis in dairy cattle in Makuyu Division Murang'a County Kenya.

### **Specific objectives**

- i) To determine prevalence of *Brucella abortus* antibodies in milk and serum from dairy cattle in Makuyu Division.
- ii) To assess the knowledge level of brucellosis among the dairy farmers in Makuyu Division.
- iii) To evaluate the accessibility of veterinary services to dairy farmers in Makuyu Division.

## LITERATURE REVIEW

The genus *Brucella* consists of species of small non-motile, non-spore forming, gram-negative aerobic coccobacilli (Dricot *et al.*, 2004). There are four types of *Brucella* species and each has a predilection for a specific host but will also infect a wide range of other animals and humans. *Brucella abortus* is for cattle, *Brucella melitensis* for goats and to a lesser extent sheep, *Brucella ovis* causes epididymitis in rams and *Brucella canis* for dogs (Harze, 2002). All the pathogenic members of the genus target cells of the host's reticulo-endothelial system, into which they lodge (Cloeckaert *et al.*, 2003). These organisms are destroyed by exposure to high temperature at 60°C for 10 minutes, exposure to phenol for at least 15 minutes or exposure to low temperatures such as at 0°C for a month (Ettunge, 1995). Their optimum temperature for growth is 37°C and the most suitable pH is 6.6-7.4 (Capasso, 2002). Some species like *Brucella abortus* are metabolisers of mesoerythritol abundant in pregnant uteri of some animals and improves *in-vivo* growth (Jubb *et al.*, 1993). *In vitro* growth is improved by addition of serum, blood or tissue extract. Serum dextrose broth and agar are ideal (Mclean, 1992). Their cell wall is made up of lipopolysaccharide-protein which maintains cell structure and provides protection from host antibodies.

Man acquires brucellosis mainly by eating or drinking contaminated milk products from infected cows, sheep and goats (Gall and Nielsen, 2004). If the milk is not pasteurized, these bacteria can be transmitted to persons who drink the milk or eat cheese made from it. *Brucella* organisms can

enter into the body through skin wounds which is a problem mainly for persons working in slaughter houses or meat packaging plants or for veterinarians. Handling new born animals or foetal membranes is a common direct method of transmission. (Makitat *et al.*, 2008). In animals transmission of *Brucella* is very likely to occur via the oral route because animals tend to lick aborted fetuses and genital discharge of the aborting animal. It is also likely to occur in utero or when calves born to healthy dams are fed on colostrum or milk from infected dams (Abbas and Aldeewan, 2009). Animals infected by *Brucella* organisms accord themselves protection by production of humoral antibodies: IgG and IgM among others, and cell mediated immunity (CMI), or both. Vaccination also offers protection by production of humoral antibodies and probably by stimulating CMI (Stephens *et al.*, 1995). The methods commonly used in diagnosis of brucellosis include Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT), Serum Agglutination Test (SAT) and Complement Fixation Test (CFT). Other available tests are Card Test, Rivanol Test, Coombs Antiglobulin Test, 2-Mercapto-ethanol (ME) and Enzyme linked Immunoabsorbent Assay (ELISA) (Faye *et al.*, 2005).

Human disease is best controlled by effective control of disease in animals (Shirima, 2005). The most important control measures against human brucellosis are pasteurization of dairy products and inspection of domestic animals for evidence of the disease. The use of goggles or face shield and rubber gloves helps protect veterinarians, butchers and slaughter house workers. Treatment

for brucellosis is by use of combinations of drugs which are tetracycline and streptomycin, doxycycline and rifampin (Dricot *et al.*, 2004). The most extensively used vaccines in control of animal brucellosis are *Brucella abortus* strain 19 and *Brucella melitensis* rev-1 (Pappal *et al.*, 2004). Test and slaughter is also effective and quick but expensive and sometimes impractical when the prevalence is high. It has however been noted that where all animals in an infected herd have been slaughtered, brucellosis does not recur.

### MATERIALS AND METHODS

Makuyu Division is divided into 12 locations. Thika-Sagana highway forms the boundary to the East, Kenol-Murang'a highway to the West and River Thara to the North. The Murang'a-Ruiru railway line passes through the division on the West. The division is served by several dry weather roads. Small scale farmers form a greater percentage of population and livestock farming is mainly on zero grazing units.

According to August 2009 census records at Makuyu livestock centre, there were 435 dairy cattle in Makuyu Division. A representative sample size of number of dairy cattle was determined using the formula deduced by Yamane (1967).

$$n = \frac{N}{1 + N(e)^2}$$

Where; n= Sample size, N= Population size, e = Level of precision at 95% confidence interval.

$$\text{Samplesize} = \frac{435}{1 + 435(0.5)^2} = 208.38$$

This study involved any lactating dairy cattle regardless of the breed and excluded non-lactating dairy cattle. This cross sectional study involved multi-stage sampling of dairy cattle in Makuyu Division. The twelve locations in the division were numbered and using systematic sampling, every third location was selected hence first, fourth, seventh and eleventh locations. Each location was divided into thirteen clusters of homesteads using cluster random sampling. From each cluster of homesteads four lactating dairy cows were selected using simple random sampling. Each lactating dairy cattle in a cluster was assigned a number, and four numbers were picked from the total randomly. Therefore a total of 208 dairy cows were used in this study. The owner of the cow was also interviewed using a questionnaire. The types of questionnaires were structured non distinguished questionnaire in which questions were listed in a pre-arranged order and the respondent told about the purpose of collecting information and non-structured non distinguished questionnaire in which questions were not structured, researcher was free to ask questions in any sequence and respondents were told about purpose of collecting information. The types of questions included closed ended questions in which the respondent selected answers from a fixed list of replies and chose any one of the multiple options given and open ended questions in which the respondent gave his or her opinion. The head of the homestead or person taking care of the cattle was interviewed and about 90% responded to the questionnaires.

Serum was screened using Rose Bengal Plate Test (RBPT) while milk was tested using Milk Ring Test (MRT) in Central Veterinary Laboratories at Kabete. Descriptive statistics was used to analyze the data collected using questionnaires to show whether veterinary services are accessible to the dairy farmers. Social data to establish relationship between levels of education and knowledge were analysed using chi-square. Level of significance was  $P < 0.05$ . Prevalence of brucellosis was analysed by use of central tendencies particularly the percentages. Percentage prevalence of brucellosis in Makuyu Division was calculated based on the number of positive animals detected using milk and serum samples.

## **RESULTS**

### **Demography of the farmers**

This study established that most of dairy farmers in Makuyu Division are above 40 years of age (76.4%). Between 31 and 40 years they were 13.5% while the rest were below thirty years. Over half of the respondents were females (56.7%). Although most of the respondents were literate over a fifth of them reported not to have had any formal education (Figure 4.1). About 60% had either attained primary or secondary school level of education.

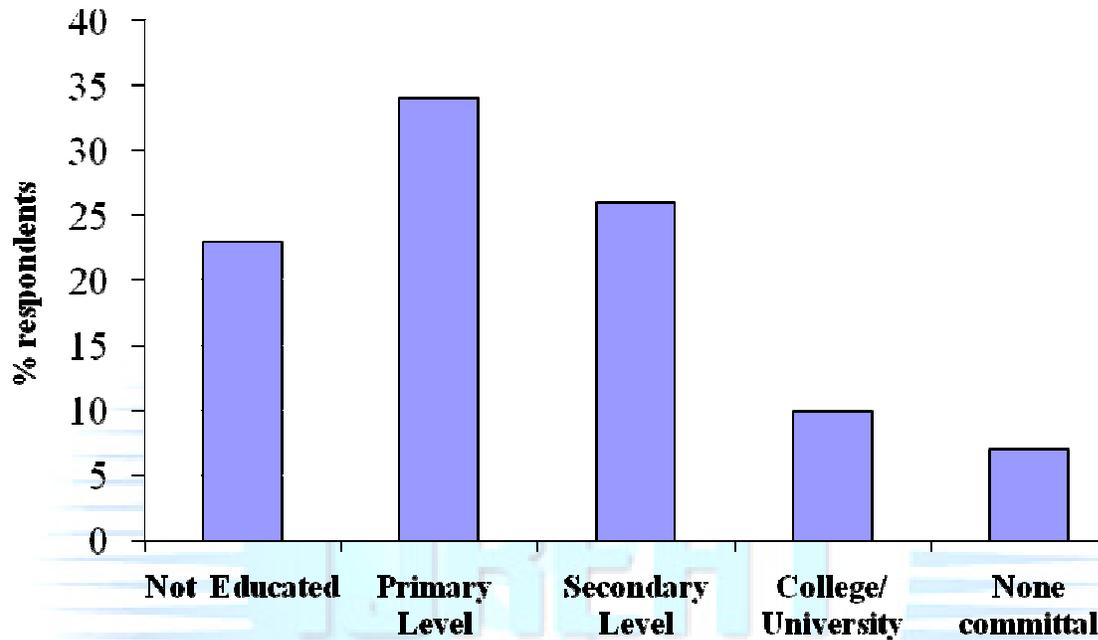


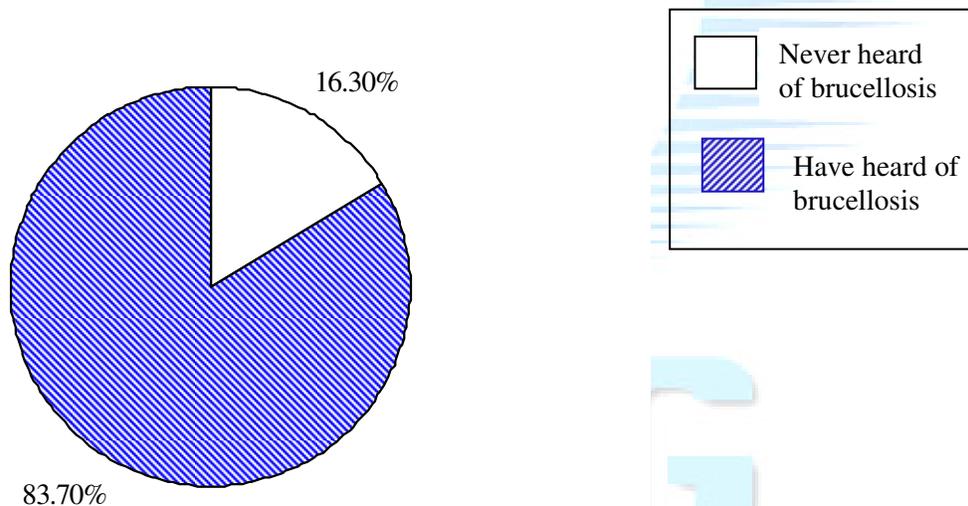
Figure 4.1: Education levels of the respondents from Makuyu Division, Murang'a County

#### Number of animals, breeds and farming practices

Since most farmers in Makuyu Division are small scale they keep less than ten animals. Friesian, Ayrshire and their classes with indigenous breeds are most common in Makuyu region. Majority (43.3%) of the farmers owned mixed breed, sixty two (29.8%) Ayrshire only while fifty six (26.9%) owned Friesian breed of cattle. Dairy farming is a labour intensive activity which generates employment, at least casual labour. In this area, one hundred and fifty three (73.6%) of the respondents practice zero grazing, forty one (19.7%) graze in an open field while fourteen (6.7%) of the farmers have paddocks in their farms where the animals are grazed.

### Knowledge of brucellosis among dairy farmers

Majority of farmers (174) reported to have heard about brucellosis (Figure 4.2). These farmers suggested the following as the main symptoms of a cattle suffering from brucellosis; reduced production of milk, swollen mammary glands, abortion, malaria like symptoms and blood stains in faecal matter. Abortion is the main symptom of brucellosis in cattle. However, most of them (83.7%) had no idea on what causes this disease but a few of them (6.7%) thought brucellosis is caused by bacteria or virus while six (2.9%) of the respondents thought it is caused by fungi. Brucellosis is caused by bacteria.



**Figure 4.2: Respondent farmer's knowledge status on existence of brucellosis in Makuyu Division, Murang'a County**

There was a significant relationship between level of education and knowledge on what causes brucellosis ( $\chi^2 = 82.542$ ;  $P < 0.05$ ). Farmers having secondary and college education are more

knowledgeable about the causes of this disease (Figure 4.3). Most of the farmers (70.2%) were aware that this disease can infect human beings and hence they are cautious when handling suspected animals and their products. The rest of the farmers also practice good hygiene in handling animals and their products such as boiling milk. Majority of the farmers (67.3%) said they have either been infected or seen a person suffering from brucellosis. The farmers strongly believe that this disease can be spread to human beings through raw milk as indicated by 63.5% of the respondents. According to the farmers, the known symptoms of brucellosis on human beings were; joint pain and body weakness, fever and headache, vomiting and backache.

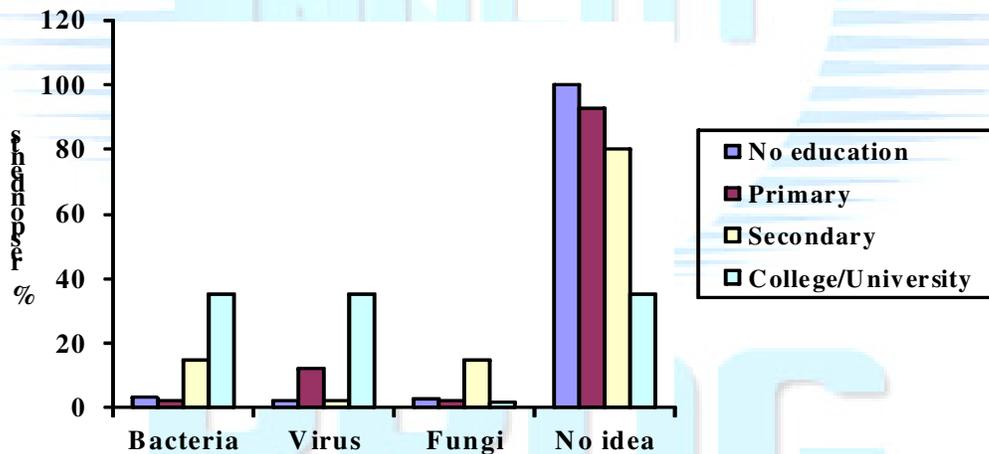


Figure 4.3: Farmers of varying education levels' views on causes of brucellosis in Makuyu Division, Muranga County

Knowledge of brucellosis was also significantly related to the age of the respondents ( $\chi^2 = 35.800$ ;  $P < 0.05$ ). Interestingly, younger farmers had heard of the disease called brucellosis more than the older respondents. All (100%) of the respondents in the age bracket of 26-30 years had heard of the disease while 75% of those in 31-40 years and 83% of those above 40 years had not heard of brucellosis.

**Table 1: Farmers knowledge on the spread of brucellosis to human beings**

Respondents ages	Knowledgeable	Not knowledgeable
26 – 30	14 (100%)	0 (0.0%)
31 – 40	14 (50.0%)	14 (50%)
Above 40	111 (69.8%)	48 (30.2%)
Chi- value	11.093	
P – value	0.004	
<b>Gender</b>		
Males	62 (74.7%)	21 (25.3%)
Females	84 (71.2%)	34 (28.8%)
Chi- value	0.302	
P – value	0.632	
<b>Education level of the farmer</b>		
No education	14 (29.2%)	34 (70.8%)

Primary	56 (80.0%)	14 (20.0%)
Secondary	55 (100%)	0 (0.0%)
College/University	21 (100%)	0 (0.0%)
Chi- value	80.94	
P – value	0.0001	

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### **Control of brucellosis**

The rate of abortion in cattle in the study area is relatively high as sixty three percent of the farmers reported that their cattle abort. They were however not aware of what exactly caused the abortion. When this occurred, about one hundred and thirty nine (66.8%) of the farmers buried the carcasses while twenty seven (13.0%) gave the carcasses to dogs. Other hygiene practices include washing hand and udder before milking, using hot water when washing, using clean milking cans and observing good hygiene of the person milking. Most of the farmers (73.1%) sell part of their raw milk to their neighbours, neighbouring shopping centres or to dairy firms. However, any milk meant for domestic consumption is boiled in two hundred and one (96.6%) of the households. Forty (19.2%) of the farmers indicated that they ferment some milk for domestic consumption.

### **Veterinary services**

Ninety one (43.8%) of the respondents knew there are veterinary doctors going around the Division. However, in some parts, the veterinary doctors only provide the services when

requested by the farmers. Most of the farmers (86.5%) reported to have had their animals vaccinated at least once in a year. Seven (3.4%) of the farmers have their animals vaccinated once in a month while another seven (3.4%) get their animals vaccinated when sick. These farmers did not indicate the disease against which their animals were vaccinated. Fourteen (6.8%) of the farmers however, failed to indicate the frequency of the vaccination indicating that they may not be vaccinating their animals. When the farmers call a veterinary doctor to attend to their animals, one hundred and eighty seven (89.9%) of the farmers realized the doctors do tell them the disease and drug used to treat the animals. In many cases (63.9%), the animals get healed after treatment (Figure 4.4). If the animal is suffering from brucellosis, the farmers suggested the following options; treat the animal, vaccinate all the animals, use artificial insemination to avoid transmission, educate farmers through extension services and slaughter the sick animal.

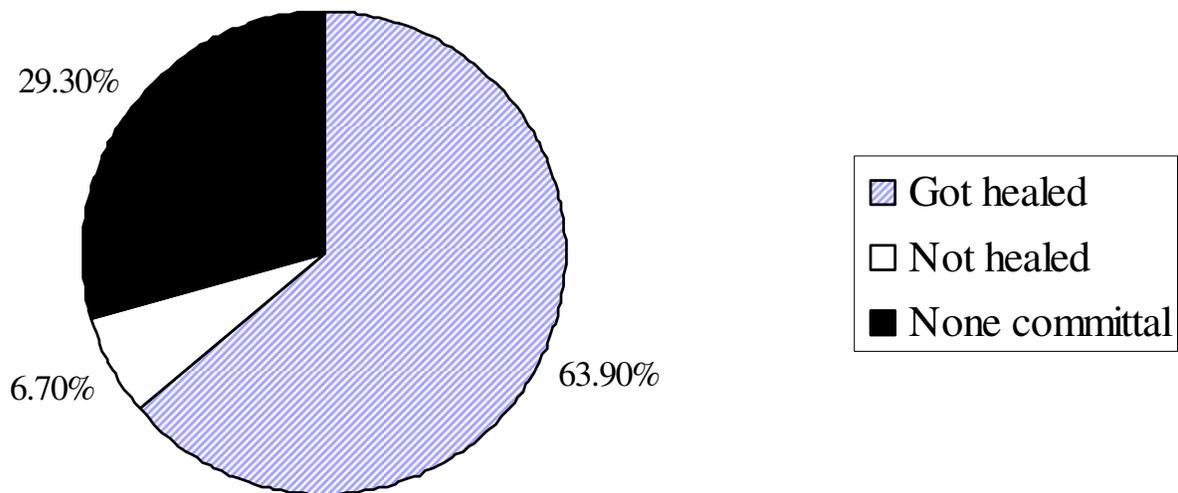
**Table 2: Accessibility of veterinary services to dairy farmers in Makuyu Division**

QUESTION	RESPONSE
Do you have veterinary personnel in this location?	43.9% knew.
Do the veterinary personnel go visiting homes in the location?	Only upon a request.

When the veterinary personnel attend to your animal, does he/she tell you the disease and drug used to treat the animal?	89.9% confirmed that the personnel inform them.
How often are your animals vaccinated?	86.5% do it once in a year.

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**Figure 4.4: Respondents reports on outcome of treatment in Makuyu Division, Murang'a County**

#### **Association between prevalence and the type of farming practices**

There was significant association in the prevalence of the disease to the farming practices carried out by the farmers ( $\chi^2 = 9.142$ ,  $P = 0.010$ ). Farmers who grazed their animals on a far field (82.9%) had experienced a sudden death of their calves while those who practiced zero grazing (17.1%) had not.

#### **Prevalence of brucellosis in Makuyu Division**

The two methods used to test brucellosis gave different results. The Milk Ring Test detected 16 cases and RBPT 4 out of the 208 animals diagnosed. This is a prevalence of 7.7% and 1.9% (Table 1). The rate of prevalence also varied with the breed of animal. Most of the animals that were infected were Fresians. Overall brucellosis was more prevalent in Fresian (13.27%) than in Ayrshire (9.43%).

**Table 3: Prevalence of brucellosis as diagnosed in milk and blood samples**

Methods	Fresian positive	Ayrshire Positive	Total percentage
Milk Ring Test	10	6	7.7%
Rose Bengal Plate Test	4	0	1.9%

Percentage prevalence of the occurrence of brucellosis in Makuyu Division, Murang'a County – Kenya was therefore calculated based on the number of positive animals detected using milk and serum samples from 208 animals.

$$\% \text{ prevalence} = \frac{\text{Positive samples}}{\text{Total number of animals}} \times 100$$

## DISCUSSION

Brucellosis is an infectious disease of non-human mammals that is contagious for man. The zoonotic disease mainly caused by *Brucella abortus* which infect cattle has severe hazards to human health (Hasanjani, 2006). In cattle the clinical signs are abortion, arthritic joints and retained after birth. It is transmitted by ingestion of *Brucella* in aborted fetuses, vaginal discharges, contaminated feeds and water and venereal transmission by infected bulls (Omer *et al.*, 2000). In man transmission is mainly by ingestion of contaminated milk and milk products (Young, 2009). Clinical signs are mild fever, weakness, enlarged lymph nodes and weight loss. Application of serological diagnostic tests for brucellosis has been achieved in diverse areas using RBPT and MRT (Kagumba and Nandokha, 1978). These two tests and questionnaires were used to obtain information on *Brucella abortus* antibodies in dairy cattle and public knowledge of brucellosis among dairy farmers in Makuyu Division.

The young and educated farmers were more knowledgeable on brucellosis than those who were old and not educated. Some of the farmers reported that when their cows abort they give the carcasses to dogs which may also have lead to increased spread of the disease. This is related to Uganda where poor disposal systems for aborted materials because of poor community knowledge about the zoonotic implications of the disease and the collapse of the animal health service sector in the rural areas as a result of privatisation of veterinary services contributed to perpetuation of the disease (Makita *et al.*, 2008). Veterinary services are available and accessible to the dairy farmers thus minimizing spread of the disease. Majority of the farmers indicated that

they do not use artificial insemination. In Egypt use of artificial insemination is encouraged as opposed to bulls to reduce prevalence of brucellosis (McDermott, 2002).

The overall prevalence of brucellosis as determined by both MRT and RBPT were 7.7% and 1.9% respectively. The *Brucella* organisms localize mainly in the lymphatic tissue, especially the supra-mammary lymph nodes, from where they are excreted via the mammary gland (Eisencheck *et al.*, 1995). The percentage prevalence of brucellosis in Makuyu Division based on milk samples was relatively high compared to others reported in some parts of the country. Kang'the *et al.* (2000) reported an overall prevalence of brucellosis at consumer-level and informal market level as 3.9% and 2.4% using MRT. Informally traded raw milk from dairy co-operatives and milk bars had the highest proportion of MRT positive samples and all these samples were from Narok District where extensively grazed pastoralist zebu herds predominate. The few cases recorded with RBPT may be due to the fact that people in this Division have small pieces of land and some of the farmers move their animals from one place to another searching for pastures. Using RBPT, Junaidu *et al.* (2008) reported prevalence of 5.1% among nomadic cattle herds and 4.4% in settled cattle in Adamawa state in Nigeria. Movement of pastoral Fulani herdsmen and interaction of cattle with those of other Fulani herdsmen is a major factor in spreading brucellosis in Nigeria.

The overall brucellosis was more prevalent in Fresian (13.27%) than in Ayrshire (9.43%) and also no antibodies were detected in the blood in Ayrshire type of breed. In Nigeria, Junaidu *et al.* (2008) reported the Sokoto Gudali breed to have the highest prevalence of brucellosis followed

by Azuwarq, with Bunaji having the least. Genetic variation is an important factor in conferring resistance or tolerance of cattle breeds to a wide range of diseases. Significant genetic variability in resistance/susceptibility to brucellosis has been detected in cattle and is associated with a 3' untranslated polymorphism in the *slc1a1* gene (Samaha *et al.*, 2008).

## CONCLUSION AND RECOMMENDATIONS

### Conclusion

- i) The point prevalence of brucellosis in Makuyu Division is estimated to be 7.7%. This shows that brucellosis occurs in Makuyu Division and therefore the human beings were exposed to the risk of the disease.
- ii) Level of knowledge of brucellosis among dairy farmers is low. It is related to their education level and age. The young who are more educated are aware of the symptoms of the disease while the old and not educated are not.
- iii) Veterinary services are accessible to the farmers, sick cattle are treated and most are healed. Veterinary personnel also inform farmers of the disease the sick animals suffer which may reduce prevalence of brucellosis.

### Recommendations

- i) Public education on methods of controlling brucellosis to reduce prevalence of the disease in dairy cattle.
- ii) Heard health campaign with frequent screening for brucellosis to identify infected animals so that they can be slaughtered to eradicate the disease.

- iii) Use of artificial insemination as opposed to bulls to control venereal transmission of brucellosis.
- iv) Future research on prevalence of brucellosis in human beings, goats and dogs.

## REFERENCES

- Abbas, B. and Aldeewan, A. (2009). Occurrence and epidemiology of *Brucella* species in raw milk samples at Basrah province, Iraq. *Bulgarian Journal of Veterinary Medicine* **12**: 136-142.
- Bruce, D. (1887). A contribution to historical understanding of brucellosis in Chile. *American Medical Association* **103**: 665-677.
- Capasso, L. (2002). Bacteria in two Millennia-old cheese, and related epizoonoses in Roman population. *Pan American Health Organization Science Publication* **45** (2): 122-127.
- Cloekaert, A., Grayon, M., Grepinet, U. and Boumedine, K. S. (2003). Classification of the *Brucella* strains isolated from marine mammals by unfrequented restriction site-PCR and development of specific PCR identification tests. *Microbes Infection*. **5**: 593-602.
- Dricot, A., Rual, J. F., Lame, P. and Beltru, N. (2004). Generation of *Brucella Melitenensis*. *Genome research* **4**: 2201-06.
- District Veterinary Office (DVO), Murang'a South, (2009). Annual report on diseases infecting cattle in Makuyu Division. 17<sup>th</sup> August 2009.
- Eisencheck, F. C., Honle, J. J. and Hoffman, E.M. (1995). Serum sensitivity of field isolates and laboratory strains of *Brucella abortus* isolates. *Veterinary Research* **56** (12): 1592-98.
- Ettunge, S. J. (1995). Text book of veterinary Internal Medicine WB Saunders Company. 654.
- Faye, B., Castel, V., Lesnoff, M., Rutabinda, D. and Dhaiwa, J. (2005). Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda). *Prevalence Veterinary Medicine* **67**: 267-281.
- Gall, D. and Nielsen, K. (2004). Serological diagnosis of brucellosis: A review of test performance and cost comparison. *Veterinary Research* **23**: 989-1002.

Gumber, S., Aradhana, N., Dhand, N. and Sondhu, K. (2004). Village level study of brucellosis in Punjab (India) by bulk milk analysis. *Indian Journal of Animal Science* **74**: 843-844.

Harze, N. D. (2002). *Brucella* virulence genome biology: The genome sequence of the facultative intracellular pathogen *Brucella melitensis*. *Infection and Immunity* **43**: 786-790.

Hasanjani, R. (2006). Efficacy of gentamicin plus doxycycline versus streptomycin plus doxycycline in the treatment of brucellosis in human clinic infectious disease. *Infection and Immunity* **42** (8): 1075-1080.

Hong, J., Elzer, p., Rowe, G. and Araya I. (2000). Epizootology of brucellosis and its relationship to the identification of *Brucella* organisms. *American Journal Veterinary Research* **25**: 553-557.

Jennings, G., Hajjeh, R., Grgis, F., Fadeel, M., Maksoud, M. and Wsfy, M. (2007). Brucellosis as a cause of a cute fibrile illness in Egypt. *Transactions of the Loyal Society of Tropical Medicine and Hygiene* **101**: 707-713.

Jubb, K. V., Kennedy, P. C. and Palmer, N. (1993). Disease of pregnant uterus, bones and joints. Pathology of domestic animals 3<sup>rd</sup> ed. Academic Press London. **3**: 396-412.

Junaidu, A., Oboegbulem, S. and Salihu, M. (2008). Seroprevalence of brucellosis in prison farm in Sokoto, Nigeria. *Veterinary Medical Association* **137**: 256-261.

Kagumba, M. and Nandokha, E. (1978). A survey of the prevalence of bovine brucellosis in East Africa. *Veterinary Microbiology* **90**: 111-134.

Kagunya, D. and Waiyaki, P. (1978). A serological survey of animal brucellosis in North Eastern Province of Kenya. *Veterinary Research* **2**: 35-40.

Kangethe, E. K., Arimi, S. M. and Omore, A. (2000). The prevalence of antibodies to *Brucella abortus* in marketed milk in Kenya and its public health implications, in proceedings of the 3<sup>rd</sup> All African Conference on Animal Agriculture.

Kang'ethe, E. K., Ekuttan, C. E. and Kiragu, M. W. (2007). Investigations into the prevalence of bovine brucellosis and the risk factors that predispose humans to infections among urban dairy and non-dairy farming households in Dagoretti. *East Africa Medical Journal* **77**: 65-69.

Makita, K., Fevre, E. and Waiswa, C. (2008). Human brucellosis in urban and peri- urban areas of Kampala, Uganda. *Applied Environmental Microbiology* **49**: 309-311.

McDermott, J. J. and Arimi, S. M. (2002). Brucellosis in Sub-Saharan Africa: Epidemiology control and impact. *Veterinary Microbiology* **90**: 1-4.

Mclean, D. (1992). Neurobrucellosis: clinical and therapeutic features. *Clinical Infectious Disease* **15** (4): 582-590.

Muriuki, S. M., Arimi, S. M. and McDermott, J. J. (1994). Brucellosis as a course of human illness in Narok District, Kenya. Proceedings of 7<sup>th</sup> Symposium on veterinary epidemiology and economics, Nairobi, 15<sup>th</sup> to 19<sup>th</sup> August 1994 Kenya Veterinary **18**: 537.

Omer, M., Skjerve, E., Holstad, G., Woldehiwet, Z. and Macmillan, A. (2000). Prevalence of antibodies to *Brucella* species and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock wildlife interface areas of Zambia. *Epidemiology and Infection* **125**: 447-4

Pappal, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tslanos, E. V. (2004). The new global map of human brucellosis. *Lancet Infectious Diseases* **6**: 91-99.

Samaha, H., Mohamed, T., Khoudair, R., Ashour, H. (2008). Sero-diagnosis of brucellosis in cattle and humans in Egypt. *Veterinary Microbiology* **78**: 56-64.

Shirima, G. M. (2005). The epidemiology of brucellosis in animals and humans in Arusha and Manyara regions of Tanzania Phd Thesis University of Glasgow.

Stephens, M. G., Olsen, S. C. and Pugh, G. W. (1995). Comparison of immune response to brucellosis in mice vaccinate with s19 or RB-51. *Immunity and Immunopathology* **63** (1): 264-270.

Yamane, T. (1967). Highly sensitive spectrophotometric kinetic determination. *Economy and environmental program* **171**: 325-332.

Young, E. J. (2009). *Brucella* species: Principles and practice of infectious disease. Churchill livingstone 226.