

Prevalence, Risk factors Associated with Brucellosis and Presence of Pathogenic Bacteria isolated from Camel Milk in Garissa County, Kenya

ABSTRACT

Aim: The current study was undertaken to assess the prevalence, risk factors of brucellosis and presence of pathogenic bacteria isolated from camel milk in Garissa County, Kenya.

Methodology: The study design was cross-sectional where questionnaires were administered to farmers to assess the risk factors associated with brucellosis. The experimental study was also employed to identify bacteria in milk samples which were collected from 104 camels. Fifty milk samples were obtained from local farms while 54 were from sales point at Garissa market. Further test for brucellosis using milk ring test was also carried out.

Results: The overall prevalence of brucellosis in camel milk was 8%. Most (12.5%) of the positive samples were from Dadaab Sub-county while the rest of the positive samples were from Fafi (5.9%) and Balambala (5.9%) sub-counties. All the 54 samples obtained from Garissa market were negative of brucellosis. Of the total (118) bacteria isolates, those from the farm level were 68.6% and from market were 31.2%. The bacteria isolated from the 104 milk samples were *Pseudomonas* spp. (32.2%), *Salmonella* spp. (30.5%), *Staphylococcus* spp (21.2%), *Eschericia coli* (8.5%) and *Shigella* spp. (7.6%). The risk factors that significantly ($p < 0.05$) associated brucellosis were: age of lactating camels (higher in camels aged above 20 years), herd size (higher in camels from herd sizes of between 30-50 camels), and herding of camels with other livestock (higher in camels kept with other livestock).

Conclusion: In conclusion, a few milk samples from camels in Garissa County were found to have brucellosis and were heavily infected with bacteria which can cause mastitis. Considering that most people in the study area drank raw milk, spread of these bacteria to man is a high possibility and thus animal and public health officers should implement one health disease control strategies.

Keywords: [Brucellosis, Camels, Milk, Bacteria, Kenya]

1. INTRODUCTION

The camel milk sector in Kenya is rapidly growing and has potential positive impacts on the livelihood of humble communities living in the arid and semiarid regions of the country. According to [1], the total production of camel milk in Kenya was recently estimated 937,000 tonnes and was valued at about Ksh 11 billion (USD 110 million). Besides the local market within the county, milk is sold to far areas like Nairobi County. However, the production of milk from the free ranging camel has several challenges including occurrence of diseases such as brucellosis and mastitis which are of economic and zoonotic significance [2].

Brucellosis is among the zoonotic diseases which have great effects on the public health of human beings in pastoral communities. The disease is usually underestimated and most of the cases are undiagnosed and untreated which creates

considerable suffering to those affected [3]. Globally, almost half a million human brucellosis cases are reported annually [4]. In a recent study by [2], prevalence of brucellosis in humans attending Ijara District Hospital in North Eastern Kenya was 15.4% showing a high exposure of brucellosis in this community. However, there are a good number of unreported cases due to non-specific clinical symptoms of the disease. The study by [2], showed that milk consumption practices were a major determinant of brucellosis risk. Besides affecting human beings, brucellosis significantly affects camels with losses being associated with treatment costs, abortion, hindered growth rate, reduced milk production and poor reproductive efficiency [5].

Camel keeping is one of the main farming activities in Garissa County, which is found in north Eastern Kenya. A few studies done in the area indicate brucellosis affect the local human population. However, there is need to document the risk factors of the disease in the animals. Further, milk is known to harbour bacteria which can not only affect the udder health of the camel but can also be of zoonotic significance thereby affecting the milk consumers. However, data on bacteria present in camel milk is scarce. Therefore, this study investigated the prevalence, risk factors of brucellosis and other pathogenic bacteria found in camel milk in Garissa County, Kenya.

2. MATERIAL AND METHODS

2.1 Study area

The study was conducted in Garissa County, Kenya, which is an arid and semi-arid region and receives an average level of 362mm of precipitation annually. The average temperature is 29.3 degrees Celsius and rainfall is 250-350mm. The county has a human population of 923,000 [6] and the area of the land is about 45,720.2km². The distance from Garissa town to Nairobi city is 360km. The camel population in Garissa County is approximately 10,000 heads while the other livestock are cattle 9,189,510, goats 1,755, 347, sheep 1,054, 822 and donkeys 67,082 [7]. Administratively, the county has 4 sub-counties, Fafi, Dadaab, Balambala, and Ijara. Fafi, Dadaab, and Balambala sub-counties were selected to participate in the study.

2.2 Study design and sample size determination

The design of the study was cross-sectional. The sample size was determined using the provided simplified formula as described by [8], where calculation was based on a population of 10,000 camels in the county and previous brucellosis prevalence of [9]. In this study, 104 camel milk samples were obtained. Fifty camel milk samples were obtained from the camels in the local farms while 54 were from sales point's at Garissa market.

2.3 Milk sample collection and handling

The lactating camel was restrained and the teats/ udder were scrubbed to remove hair and dirt. Teats and udder were then washed using cotton wool soaked with 70% ethanol. Using sterile soft piece of towel, the teats were dried. The teats were then pressed to discard the first two streams of milk. Ten milliliters of milk were collected from the four quarters of the 50 lactating camels into sterile universal tubes. Further, 54 camel milk samples were obtained from the point of sales at the Garissa market. Milk samples were then labelled put into a cool box and transported to the Jomo Kenyatta University of Agriculture and Technology Microbiology laboratory.

In the grouping, camels were grouped according to stages of lactation and these formed three groups (1st lactation, 2nd and 3rd lactation), age of lactating camel, parity, and herd size.

2.4 Determination of presence of brucellosis in camel milk

Milk ring test was undertaken as described by [10]. Briefly, 0.03mL of antigen *B. abortus* and *B. melitensis* from Fortress Diagnostics Limited was added to 1mL of raw camel milk which had been stored at 4°C for 24 hours. The milk samples were then incubated for an hour at 37°C, a control sample was also subjected to similar conditions. A positive reaction was indicated by the formation of a dark blue ring above the white milk column. The test was considered to be negative if the colour of underlying milk remained homogeneously dispersed in the milk column [10].

2.5 Determination of other bacterial microorganisms present in camel milk

Each camel milk sample was cultured in six different agars (Nutrient agar, Mannitol salt agar, *Salmonella-shigella* agar, EMB agar, pseudomonas isolation agar, and McConkey agar). Cultures were put in an incubator for 24 hours at 37°C. The growth of the cultures was examined and the bacteria were classified based on their colonial and gram staining characteristic. The identification of the genera of the isolated bacteria was based on biochemical characteristics such catalase, coagulase and oxidase tests.

2.5 Questionnaire survey

The questionnaires consisted of a series of questions and were administered to pastoralists at farm levels in Garissa County to document information on biodata, number of camels kept, brucellosis related questions and the risk factors of the disease. The risk factors associated with the prevalence of the disease which were assessed in the questionnaire were knowledge of brucellosis occurrences, age, herd size, parity, consumption of unpasteurized milk, ways of disposal of placenta, breeds, cases of retain afterbirth, interaction with other livestock amongst other farm management factors e.g. vaccination.

2.6 Data analysis

Data was entered into Microsoft-Excel spreadsheet and thereafter exported to Software Package for Social Science (SPSS) where it was analysed. Descriptive statistics were computed for the milk ring test and laboratory culture results to establish the frequencies and percentages. A chi-square test was used to assess association between risk factors and brucellosis ($p < 0.05$). For the purpose of this study, 95% probability was considered as a significant confidence level.

3. RESULTS

3.1 Characteristics of the camel farms and the farmers

A total of 50 farmers were selected randomly from Dadaab (16), Fafi (17), Balambala (17) sub-counties of Garissa County. The demographic characteristic of farmers and sampled camels are shown in Table 1. Most (86.0%) of the house-hold heads were males while only 14.0% were female. Majority (66.0%) of the sampled farmers had attained non-formal education and only (8.0%) had attained tertiary education. Most (66.0%) of the farmers kept camels with other livestock. Majority (60.0%) of the sampled farmers owned 20-30 camels while a few kept owned 50-100 camels (12.0%). Ninety per cent of the sampled farmers consumed milk without any heat treatment due to beliefs that camel milk is nutritious and medicinal when consumed raw.

Ninety per cent (90%) of the farmers did not dispose the placenta material of the camel after parturition and leaving it in the open fields. Only 10% buried the camel placenta in the ground. The losses experienced by farmers as a result of camel brucellosis included abortion (46%), reduced milk production (16%) and infertility (8%). Farmers whose camels experienced cases of retained afterbirth were 50%. Most farmers (64%) indicated that they had direct contact with afterbirth or abortion material. The latter occurred during the process of removing the after birth and disposing of the placenta.

Table 1. Socio-demographic characteristics of respondents in Garissa County, Kenya

Characteristic	Characteristic	Frequency	Percentage
Gender	Male	43	86.0
	Female	7	14.0
Level of education	Non formal	33	66.0
	Primary	7	14.0
	Secondary	6	12.0
	Tertiary	4	8.0
Preparation of milk before consumption	Consumed without boiling	45	90.0

	Pasteurization before consumption	0	0.0
	Ferment without boiling	5	10.0
Vaccination against brucellosis	Yes	0	0.0
	No	50	100.0
Disposal of placenta	Yes	45	90.0
	No	5	10.0
Contact with afterbirth/ abortion material	Yes	32	64.0
	No	18	36.0
Keeping of camels/other livestock	Camels only	17	34.0
	With other livestock	33	66.0
Losses experienced as a results of brucellosis	Abortion	23	46.0
	Reduced milk production	8	16.0
	Infertility	4	8.0
	None of the above	15	30.0
Cases of retained afterbirth	Yes	25	50.0
	No	25	50.0
Knowledge on brucellosis occurrences	Yes	30	60.0
	No	20	40.0

3.2 Prevalence of brucellosis

Milk ring test was used to examine the prevalence of camel milk brucellosis in the study area. Out of the 50 camel milk samples from the field, a total of 4 (8%) samples were positive of brucellosis. Most (2/16, 12.5%) of the positive samples were from Dadaab Sub-county while the rest of the positive samples were from Fafi (1/17, 5.9%) and Balambala (1/17, 5.9%) sub-counties. All the 54 samples obtained from sales points in Garissa market were negative of brucellosis.

3.3 Bacteria isolated from the camel milk

The total number bacterial isolates were 118. Of these bacteria isolates, 68.6% were from milk sourced from farm level while 31.4% were from the market (Table 2). In overall, most of bacterial isolates (from either field or market camel milk) were *Pseudomonas* spp. (32.2%), followed by *Salmonella* spp. (30.5%), *Staphylococcus* spp. (21.2%), *E. coli* (8.5%) and the least were *Shigella* spp (7.6%) (Table 2). From the milk samples obtained from the market the most common isolates were *Staphylococcus* spp., *Pseudomonas* spp. and *E. coli*. The most common bacteria in milk from camels sampled from the field were *Pseudomonas* spp., *Salmonella* spp., and *Staphylococcus* spp.

Table 2. Distribution of bacteria isolates in camel milk samples based on sources

Bacterial isolates	Source of milk		Percentage (%)
	Farm	Market	
<i>Escherichia coli</i>	1 (1.2%)	9(24.3%)	10 (8.5%)
<i>Staphylococcus spp.</i>	15(18.5%)	10 (27.0%)	25 (21.2%)
<i>Pseudomonas spp.</i>	29(35.8%)	9(24.3%)	38 (32.2%)
<i>Salmonella spp.</i>	29(35.8)	7(18.9%)	36 (30.5%)
<i>Shigella spp.</i>	7(8,6%)	2(5.4%)	9 (7.6%)
Total	81 (68.6%)	37(31.4%)	

3.4 Relationship between prevalence of brucellosis and risk factors (Table 3)

Age of lactating camels was found to be significantly ($p=0.004$) associated with occurrence of brucellosis where the highest (37.5%) prevalence of brucellosis was found in camels above 20 years and the lowest (7.6%) prevalence was found in camels age between 10-20 years. The herd size was found to be significantly ($P=0.001$) associated with prevalence of brucellosis with the highest prevalence being observed in camels from herd size of between 30 and 50 (23.1%) and no case of brucellosis was recorded in camels from herd size of 20 -30 camels. The lowest prevalence of camel milk brucellosis was found in the first parity (5.6%) and an increase ($p=0.76$) in prevalence was noted as the parity increased. The stage of lactation was not ($p=0.07$) associated the occurrence of brucellosis. The prevalence of camel milk brucellosis was found to be significantly ($p=0.004$) high (11.8%), in camels kept with other livestock.

Table 3. Effect of various risk factors on prevalence of brucellosis in camels as identified by milk ring test in the study area

Risk factor	N	Positive /%
i) Age of lactating camel		
4-6 years	15	0 (0.0%)
6-10 years	14	0(0%)
10-20 years	13	1(7.7%)
Over 20 years	8	3(37.5%)
ii) Parity		
1	18	1(5.6%)
2	24	2(8.3%)
3	4	1(25.0%)
More than 3 times	4	0(0.0%)
iii) Stage of lactation		
Early	10	0(0.0%)
Mid	23	1(4.3%)
Late	17	3(17.6%)
iv) Herd size		
20-30	31	0(0.0%)

30-50	13	3(23.1%)
50-100	6	1(16.7%)
v) Herd structure		
Camels only	16	0(0.0%)
Camels with other livestock	34	4(11.8%)
vi) Disposal of placenta		
Yes	45	1(0.0%)
No	5	3(0.6%)
vii) Case of retained afterbirth		
Yes	25	4(0.2%)
No	25	0(0.0%)
viii) Vaccination		
Yes	0	0(0.0%)
No	50	4(8.0%)
ix) Consumption of milk		
Unpasteurized milk	45	4(8.9%)
Pasteurized milk	0	0(0.0%)
Ferment without boiling	5	0(0.0%)
x) Breed		
Locals	50	4(8.0%)
Imported	0	0(0.0%)

UNDER

3.5 Relationship between risk factors and occurrence of other bacteria in raw camel milk from farm level (Table 4)

Higher prevalence of *Staphylococcus* spp. was found in camels having parity of 3 times (50%), lactating camels of 10-20 years (46.2%), in their early stage of lactation (50%). Further higher prevalences of *Staphylococcus* spp. was found in camels of 20-30 herd size and in camel-only herds (Table 4).

The occurrence of *E. coli* was higher in camel which were of 6-10 years of age (7.1%), originating from herd sizes of between 50-100 camels (16.7%), in mid stage of lactation and in camel-only herds. The highest occurrence of *Salmonella* spp. was found in camels having three times parity (75.0%), aged 6-10 years (85.7%), early stage of lactation (100%), 50-100 herd sizes (66.6%) and camel-only herds (62.5%).

The occurrence of *Pseudomonas* spp. was increased as parity increases. Camels with the highest prevalences were those aged 6-10 years (78.6%), in early lactation (90%), from 50-100 herd sizes (66.7%) and where camels were kept with other livestock (58.8%). The occurrence of *Shigella* spp. was found to be higher in camels of over 3 times of parity (75.0%). For the other risk factors, the occurrence of *Shigella* spp. was higher in camels over 20 years of age (37.5%), in early stage of lactation (20%), from 50-100 herd sizes and those where camels are kept in close contact with other livestock (17.7%) (Table 4).

Table 4. Relationship between risk factors and occurrence of bacteria isolates in camels (n=50) from Garissa County

Risk factor	Category	<i>Staphylococcus</i> spp. (%)	<i>E. coli</i> (%)	<i>Salmonella</i> spp. (%)	<i>Pseudomonas</i> spp. (%)	<i>Shigella</i> spp. (%)
Parity	1	11.1	0.0	44.4	44.4	5.6
	2	41.7	4.2	66.7	62.3	12.5
	3	50.0	0.0	50.0	75.0	0.0
	Over 3 times	25.0	0.0	75.0	75.0	75.0
Age of lactating camel	4-6 years	20.0	0.0	46.7	53.3	13.3
	6-10 years	35.7	7.1	85.7	78.6	7.1
	10-20 years	46.2	0.0	53.9	53.9	7.7
	Over 20years	12.5	0.0	37.5	37.5	37.5
Stage of lactation	Early	50.0	0.0	100.0	90.0	20.0
	Mid	34.8	4.4	56.5	60.9	13.0
	Late	11.8	0.0	35.3	35.3	11.8
Herd size	20-30	29.0	0.0	54.8	54.8	9.7
	30-50	23.1	0.0	61.5	61.5	15.4
	50-100	18.8	16.7	66.6	66.7	33.3
	Over 100	0.0	0.0	0.0	0.0	0.0
Consumption of milk	Unpasteurized	30.0	2.0	58.0	58.0	14.0
	pasteurized	0.0	0.0	0.0	0.0	0.0
Keeping of camels and other livestock	Camels only	31.2	6.3	62.5	56.3	6.3

	Camels with other livestock	29.4	0.0	55.9	58.8	17.7
Vaccination	Yes	0.0	0.0	0.0	0.0	0.0
	No	30.0	2.0	58.0	58.0	14.0
Breed	Locals	30.0	2.0	58.0	58.0	14.0
	Imported	0.0	0.0	0.0	0.0	0.0

4 DISCUSSION

The current study was geared towards evaluating the prevalence of brucellosis and bacteria isolated from camel milk emanating from camels at farm and market level. The overall prevalence of camel milk brucellosis in Garissa County was 8% and varied according to the sub-county of origin. Previous studies reported a prevalence of brucellosis in camels kept extensively by pastoralists as ranging between 2% and 5% [12, 13]. The slightly higher prevalence of brucellosis in Dadaab as compared to Fafi and Balambala sub-counties could be due to the fact that the former borders Somalia where there are large migration patterns of camel pastoralists. Improved control of border migration and vaccination of both small ruminants and camels were previously reported as necessary to bring down the prevalence of camel milk brucellosis in North Eastern Kenya [11]. In this study, the farmers reported high level of abortion and several cases of infertility and reduced milk production in their camels. Similar findings were reported by [13, 14] showing that the disease is of economic importance and affects the livelihoods of the farmers. Thus, its control should be prioritized. All farmers did not carry out brucellosis vaccination programmes and thus most of the camels in this area are susceptible to the disease. Although the study did not investigate the aetiology of brucellosis, previous reports suggest that camels can be infected by *Brucella abortus* and *Brucella melitensis* [12].

According to the current study, most brucellosis cases were in camel above 20 years meaning that as the camel gets older it becomes more susceptible to brucellosis. Prolonged exposure of brucellosis in aged camels could also cause high burden of the disease [15]. A high prevalence of brucellosis was observed in camels from the farms which had over 30 camels indicating that herd size is an important risk factor for the prevalence of brucellosis. A high seropositivity of brucellosis has been observed where domestic animals are kept extensively in large number [16]. This can be attributed to large herds providing more chances of contact between camels, especially during the calving season [13].

The present study showed that most of the farmers kept camels with other livestock especially sheep and goats. As reported by [17], there was an increase in prevalence of brucellosis in farms where camel intermingled with other livestock. [18], also observed higher prevalence in camels that were in contact with sheep and goats in the pastures and water points [19]. Camels are susceptible to *Brucella mellitensis* though they are not the definitive host and can acquire the disease through either direct contact with infected small ruminants or indirect contact with aborted materials from infected small ruminants.

The study showed an association between the parity and the prevalence of brucellosis where those she-camels with more than one parity were more at risk of being positive to brucellosis. These findings are consistent to those of a previous study by [20], where higher reactor rate was recorded in camels with more than one parity number as compared to single parity. Animals with one or two parturitions have high milk production which serves as a media for *Brucella* spp. Camels in the late lactation stage had a higher prevalence of camel milk brucellosis compared to those in mid and early lactation and this is similar to a previous study by [21].

The bacteria isolated from the raw camel milk samples of the camel included *Pseudomonas* spp, *Salmonella* spp, *Staphylococcus* spp, *E. coli* and *Shigella* spp. Similar profile of bacteria has been reported in camels from Algeria [22]. The predominant bacterium was the *Pseudomonas* spp and one of the species (*Pseudomonas aeruginosa*) has been shown to cause mastitis in camels. The fact that most of *E. coli* isolates were observed in market samples shows possibility of poor hygiene of milking environment and the camel milk handling [23]. A study by [24], found out that 66% of raw camel milk samples had microbial load of less than 105 cfu/mL at the site of production compared to the 54% at the market site where the microbial was over 105 cfu/ml. In Kenya, the common means of transport of raw camel milk from the production sites to the consumer or marketing site are bicycles, donkeys and vehicles in a high ambient temperature. Further, the raw camel milk reaches the bulking sites in 2-3 hours and to bigger markets in 4-5 hours. In such an environment, bacteria present in raw camel milk will multiply and reach high doses by the time it gets to the consumer [24]. It is therefore recommended to transport milk in low temperature containers such as cool boxes, or subject camel milk to pasteurization process in order to reduce the microbial count.

In the present study, the incidence of *Salmonella* spp. was higher than that reported by [25]. The reason leading to high prevalence of *Salmonella* spp in raw milk is because of the *Salmonella* spp is shed in faeces which in turn contaminates the udder and teat of camel as it sleeps. In the process of milking, the raw camel milk is contaminated with *Salmonella* spp resulting from soiling of udder [26]. *Salmonella* spp cause chronic mastitis and abscesses in animals. Further, *Salmonella* species such *Salmonella tyhimurium* is a major economic and public health threat [27, 28].

The study reveals that *Staphylococcus* spp was highly prevalent in the milk samples. [25] reported that a high prevalence (70%) of this bacteria in milk samples of camel from Ethiopia and Kenya (36%) [29]. Even in other domestic ruminants, *Staphylococcus* spp. is predominant in the milk [30]. The high burden of *Staphylococcus* spp. could be due to poor hygienic practices which mainly cause sub-clinical mastitis and major economic losses. *Staphylococcus aureus* has been shown to have a high tolerance to salt and can grow in dairy products, and the toxins produced by this bacteria are heat resistant and cannot be destroyed through cooking. Thus, *Staphylococcus* spp is zoonotic and have public health significance in humans [31].

Shigella spp. was isolated in the raw camel milk at both the farm and market level. According to [32], a prevalence of 1.4% of *Shigella* spp was observed in raw camel milk from Eastern Ethiopia. In a previous study, *Shigella* spp. was isolated from raw camel milk samples (20%) [33]. The prevalence of *Shigella* spp in milk from tanks and output milking machine was higher (80.7%) than in milk from hand milking (16.5%) and groceries (2.8%) [33]. The higher percentage of *shigella* in milking machine maybe due to use of too much water to clean the equipment. In humans, consumption of raw camel milk contaminated with *Shigella* spp can lead to shigellosis which is characterized by abdominal cramping, blood and mucus in stool and thus is a public health threat [34, 35].

The present study showed that the risk factors for presence of bacteria include parity, age of lactating camel, stage of lactation, herd size, consumption of milk, and keeping of camels with other livestock. According to the study, there was an increase in the prevalence of bacterial isolates with increase in parity. Camels which had given birth three times and more showed high percentage of bacterial isolates [20], most likely due to higher exposure with time. Similarly, as observed in this study, prevalence of the bacteria increased with age and this has been reported before [36]. [12] also reported that large herd size increases chances of contact between camels or small ruminants whereby there is transfer of pathogenic bacteria across the species. Keeping of camels with other livestock serves as a possible risk factor since other ruminants grazed together with camels can act as reservoirs for pathogenic bacteria. [37], reported that contacts between the sheep and camels especially during calving season facilitate transfer of bacteria between the host species.

The results from this study depicts that *E. coli* was higher in the market level than farm level which could mean that milk could have been transported using unclean containers. [38], showed that milk transported from the producer to the consumer using unclean plastic containers and without any form of treatment can lead to massive contamination by several pathogenic bacteria reported in the current study. The milk handling equipment should therefore be subjected to thorough cleaning to reduce the number of microbes

4. CONCLUSION

The results of this study showed that brucellosis and a variety of bacteria are prevalent in camels from the Garrisa County. Brucellosis is known to affect humans in the study area and domestic animal health [2]. The risk factors for occurrence of brucellosis in camel were age of lactating camels, stage of lactation, parity, herd size and interaction with other livestock. The results from the present study also indicate that the microbial quality of camel milk at farm and market level in Garissa County is low and will require pasteurization before consumption. This is critical because most respondents in this study indicated that they drank the milk raw, thus exposing them to the observed bacteria. The bacterial isolates included *Staphylococcus* spp, *E. coli*, *Salmonella* spp., *Shigella* spp. and *Pseudomonas* spp. These bacteria can cause mastitis to the camels and are of zoonotic significance. Therefore, strict milk hygiene control measures from production to consumption should be implemented by the farmers as advised by local extension and public health officers.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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ABBREVIATIONS

- CFU** Colony Forming Units
EMB Eosin Methylene Blue
SPSS Software Packages for Social Science