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Seroprevalence of Leptospirosis in Cattle in Smallholder Livestock Production Systems in Bungoma County, Kenya

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ABSTRACT

Leptospirosis is an important re-emerging bacterial zoonosis of public health importance in Kenya. It is transmitted through contact with contaminated soil, water, or urine of infected animals. The disease is associated with high economic losses which include high cost of diagnosis and treatment, disruption of international trade in animals and animal products and loss of milk production following mastitis among others. In this study, the sero-prevalence of bovine leptospirosis in Kimilili and Mt Elgon Sub-Counties of Bungoma County in Kenya was estimated. A cross-sectional study was conducted between April to July, 2017 where blood serum samples from 200 head of cattle from five wards and one slaughter house using systematic random sampling technique. The samples were then subjected to the Enzyme Linked Immunorsorbent Assay (ELISA) test. The findings show an overall sero-prevalence for leptospirosis in cattle in the study area to be 16%. The sero-prevalence of leptospirosis in the study area by wards is as follows; 10.7% in Kibingei, 12.5% in Kimilili, 17.6 in Maeni, 16.7% in Kamukuywa and 15.6% in Kaptama. The study also reveals that the sero-prevalence was relatively higher in Kimilili Slaughterhouse which recorded 21.0%. The study therefore demonstrates that there is a high sero-prevalence of leptospirosis in cattle in Kimilili and Mt Elgon Sub-Counties, Bungoma County. This study therefore provides critical public health information which is necessary in guiding leptospirosis prevention and control in Bungoma County. It is therefore recommended that strategies for surveillance and laboratory diagnosis of leptospirosis should be initiated in Bungoma County. This will help in providing better estimates of leptospirosis burden in the county.

Keywords: *Cattle, Enzyme-Linked Immunosorbent Assay (ELISA), Leptospirosis, Sero-Prevalence, Zoonosis*

I. INTRODUCTION

Leptospirosis is an important bacterial zoonosis which is largely neglected and over-looked in Africa (Allan *et al*., 2015). It is caused by bacteria of the genus *Leptospira.* Leptospirosis causes a wide range of symptoms in humans such as fever, chills, headache, muscle ache, jaundice, abdominal pains, diarrhea and rashes (Nakeel *et al*., 2016). These symptoms to those seen in other flu-like diseases such as brucellosis, dengue fever, malaria, hepatitis, typhoid, streptococcal infections and rheumatism (Esteves *et al*., 2018). In most cases, the infe cted persons do not show any clear and definitive symptoms at all. The lack of pathognomonic signs of leptospirosis means that diagnosis is tentatively based on evaluation of fever and myalgia in patients presenting at the hospital (Lisa *et al*., 2018). In livestock, the disease has a major impact on the reproductive efficiency which is associated with abortion, stillbirth and birth of weak neonates with a high death rate (Simegnew, 2016).

Global re-emergence of *leptospirosis* has been associated with environmental and socio-economic factors including high rainfall, flooding, poverty, urbanization, and ecotourism (Center for Disease Control [CDC], 1995; Ricaldi, 2006; Lau *et al*., 2010). *Leptospirosis* is a disease of increasing global concern and is most prevalent in tropical climates where people and animals live in close contact (Anon, 2005; Alan *et al*., 2011). It is considered the most common zoonosis and an emerging disease whose incidence is increasing, as a consequence of global climate change (Plank and Dean, 2000; Lau *et al*., 2010).

Leptospirosis is underestimated and under-diagnosed in many countries because of difficult clinical diagnosis and lack of diagnostic laboratory services (Plank and Dean, 2000; Lisa *et al*., 2018). Surveillance therefore provides the basis for any intervention strategies in human and veterinary public health (WHO/CDC, 1999).

In Kenya, the disease has been reported in both livestock and humans since 1950s (Burdin and Froyd, 1957), 1960s (Forrester *et al*., 1969) and in 2004 (Anon, 2005). The World Health Organization (WHO) and Global Alert and Response System reported a serious outbreak at Chesamisi and the surrounding areas of Kimilili and Kaptama wards in Bungoma County, Western Kenya in 2004 with 141 cases and 8 deaths reported (WHO Leptospirosis in Kenya, 2004). The outbreak is recorded as one of the disasters in Kenya and Chesamisi High School and Chesamisi Primary School were seriously affected (Government of Kenya [GoK], 2018). There was also a confirmed case in cattle in Ziwa, Uasin Gishu County in 2007 (VIL Eldoret Annual Report, 2007).

The overall objective of the study was to estimate the sero-prevalence of *leptospirosis* in cattle in Kimilili and Mt Elgon Sub-Counties, Bungoma County, Kenya.

II. METHODOLOGY

2.1 Study Site

The study was conducted in Kimilili and Mt. Elgon sub-counties in Bungoma County in the western Kenya Lake Victoria Basin. The study area included four wards in Kimilili (Kimilili, Kibingei, Maeni and Kamukuywa) and Kaptama ward in Mt. Elgon sub-counties (Figure 1). Bungoma County is located on the southern slopes of Mt. Elgon and borders Uganda to the northwest, Trans-Nzoia County to the northeast, Kakamega County to the east and southeast, and Busia County to the west and southwest. The county lies between latitude $0^0 28^0$ and $1^0 30^0$ North and longitude $34^0 20^0$ and $35^0 15^0$ East (Bungoma County Government, 2012).

The study area was selected based on the previous outbreak of human leptospirosis in 2004 in Chesamisi area of Kimilili sub-county. A critical review of hospital records at Bungoma, Kimilili, Webuye and Kaptama health facilities revealed that the cases were majorly reported in all the wards of Kimilili Sub-County and parts of Kaptama Ward in Mt Elgon Sub-County.

2.2 Study Population

More data was collected from the most active slaughter facilities in Bungoma County which comprised Kimilili and Bungoma slaughterhouses.

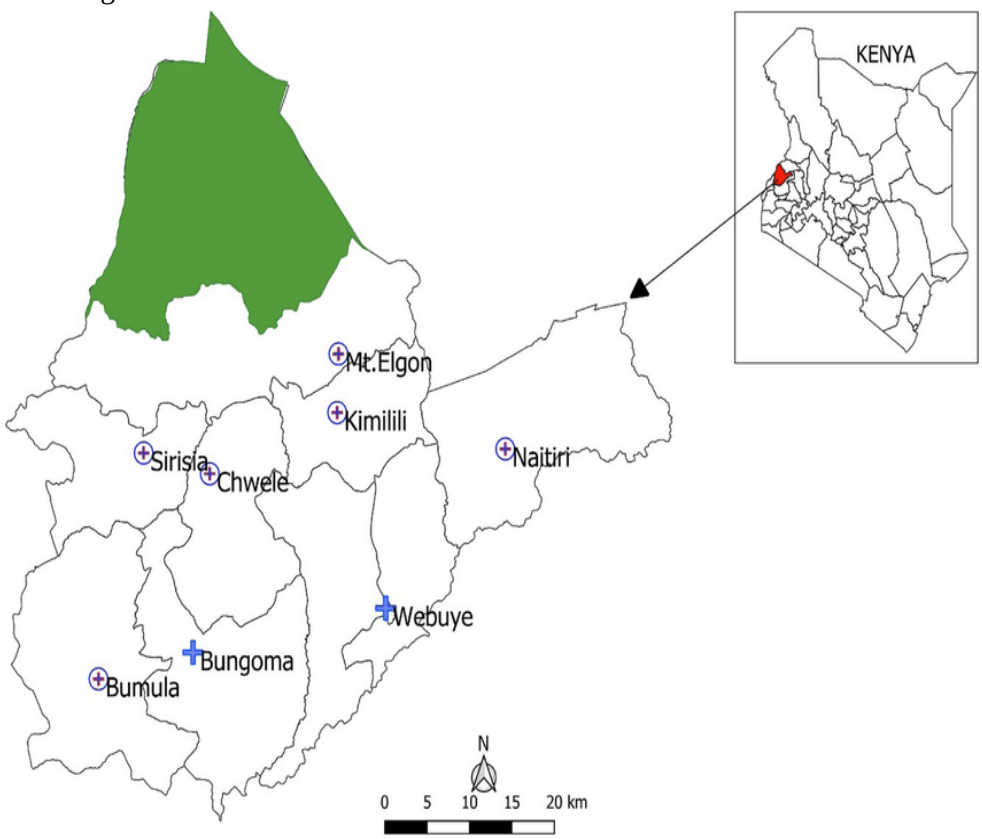

Figure 1
Map of Bungoma County in Relation to Kenya (Shaded red)
Source: KNBS, (2013)

2.3 Sample size

It's a cross-sectional survey and a minimum sample size of 178 cattle was calculated using the formula of estimating disease prevalence according to Thursfield (2005): $Z^2 \times P \times (1-P)/d^2$ where Z=1.96 and d =0.05 at 95% confidence interval. A previous study by Cook *et al.*, (2017) showed a sero-prevalence of 13.4% and therefore P=0.134 and 1-P=0.866.

2.4 Sampling procedure

Systematic random sampling method was used. The wards were stratified into locations and further into sub-locations. The sub-locations were selected purposively based on the level of previous exposure to the outbreaks. With the help of community leaders, the study team visited the selected sub-locations and the most affected households further selected purposively with the help of community leaders.

2.5 Data Collection

The study utilized both secondary and primary data. Primary data was collected from the field and secondary data from archival sources.

2.5.1 Primary Data

The total number of households and blood samples collected by location, ward and sub-county is shown in Table 1.

Table 1

Blood Samples Collected by Sub-County, Ward and Location

SUB-COUNTY	WARD	LOCATION	No. of HH SAMPLED	No. OF BOVINE SERA COLLECTED
Kimilili	Kibingei	Kibingei	6	6
		Kitayi	4	5
		Chebukwabi	10	8
		Kamusinga	16	9
	Kimilili	Kimilili	22	12
		Bituyu	10	10
		Khamulati	12	10
		Kimilili slaughterhouse	0	38
	Maeni	Kibisi	20	8
		Sikhendu	22	8
		Nasusi	29	9
		Kamasielo	31	9
	Kamukuywa	Makhonge	16	5
		Mapera	18	7
		Mbongi	24	8
		Nabikoto	18	6
		Musebe	16	5
		Kimakwa	16	5
Mt Elgon	Kaptama	Kaborom	22	6
		Kaptama	18	5
		Chesito	13	4
		Kongit	12	3
		Kaboiywo	13	4
		Kaptelelio	10	3
		Chemoge	10	3
		Chemuses	6	2
		Chemuses	6	2
			400	200

2.5.2 Secondary data

Secondary data was sourced from journals and reports from relevant offices. Literature search was carried out in relation to recent leptospirosis outbreaks in the world and recent sero-prevalence studies in livestock. The required information was obtained through the website search engines, publication databases and online libraries. Internet accessible journal and other relevant electronic publications were used. Other relevant documented materials which could not be accessed through the internet were collected from relevant offices and libraries.

The reports utilized in the study included the following;- hospital records, research theses, newspapers in the relevant areas, monthly and annual reports from the Veterinary Department and the Ministry of Health. Laboratory reports from the Regional Veterinary Investigation Laboratory (VIL) - Eldoret were also reviewed. Medical records for 1990 to 2010 were obtained from Kimilili and Webuye health facilities in Bungoma County.

2.5.3 Serum Collection and Laboratory Testing

Blood was collected by bleeding cattle from the jugular vein following restraint. Plain vacutainners and 1.5 inch needles, gauge 18 were used to collect 10-15ml of blood. The samples were labeled immediately and stored on ice in a cool box.

The blood sample was left to stand overnight in the cool box packed with ice to allow serum separation. Serum was then harvested by decanting into 2ml sterile vials and labeled appropriately and stored in a freezer at -20^{o}C. The samples were then transported to the Regional Veterinary Investigations Laboratory, Eldoret for temporary storage in a deep freezer. After collecting all the samples, they were packed with ice in a coolbox and transported to the Central Veterinary Laboratory in Kabete, Nairobi where they were tested using ELISA test. The optical densities (OD) were measured at 450 nm in a micro plate photometer (Hum reader, model 18500/1, Awareness Technology inc. Germany). Sera and controls were run in duplicate to compare the two OD reading for every sample. The appearance of a blue solution indicates the presence of *Leptospira* antibodies (positive) which then turns yellow upon addition of a stop solution. Absence of antibodies (negative results) was denoted by lack of coloration.

The sero-prevalence for *Leptospirosis* in cattle was calculated by getting the number of positive samples to ELISA test divided by the total number of samples collected and multiplied by 100%.

2.5.4 GPS Mapping of the Study Sites

Using a global Positioning System (GPS) hand held receiver, an accurate location for each of the sites visited was recorded capturing the various learning institutions that were affected by the disease in 2004, livestock markets, slaughter facilities, water bodies, health facilities that were used for the management of cases and veterinary offices. The Geo-reference data included the latitude, longitude and the altitude. The GPS was positioned to have a clear view of the sky and away from any obstructions. The readings were obtained within 3-4minutes.

Figure 2 shows the location of households visited during data collection. It also shows the health facilities where majority of patients suspected to have *leptospirosis* infection were treated during the *leptospirosis* outbreak of 2004.

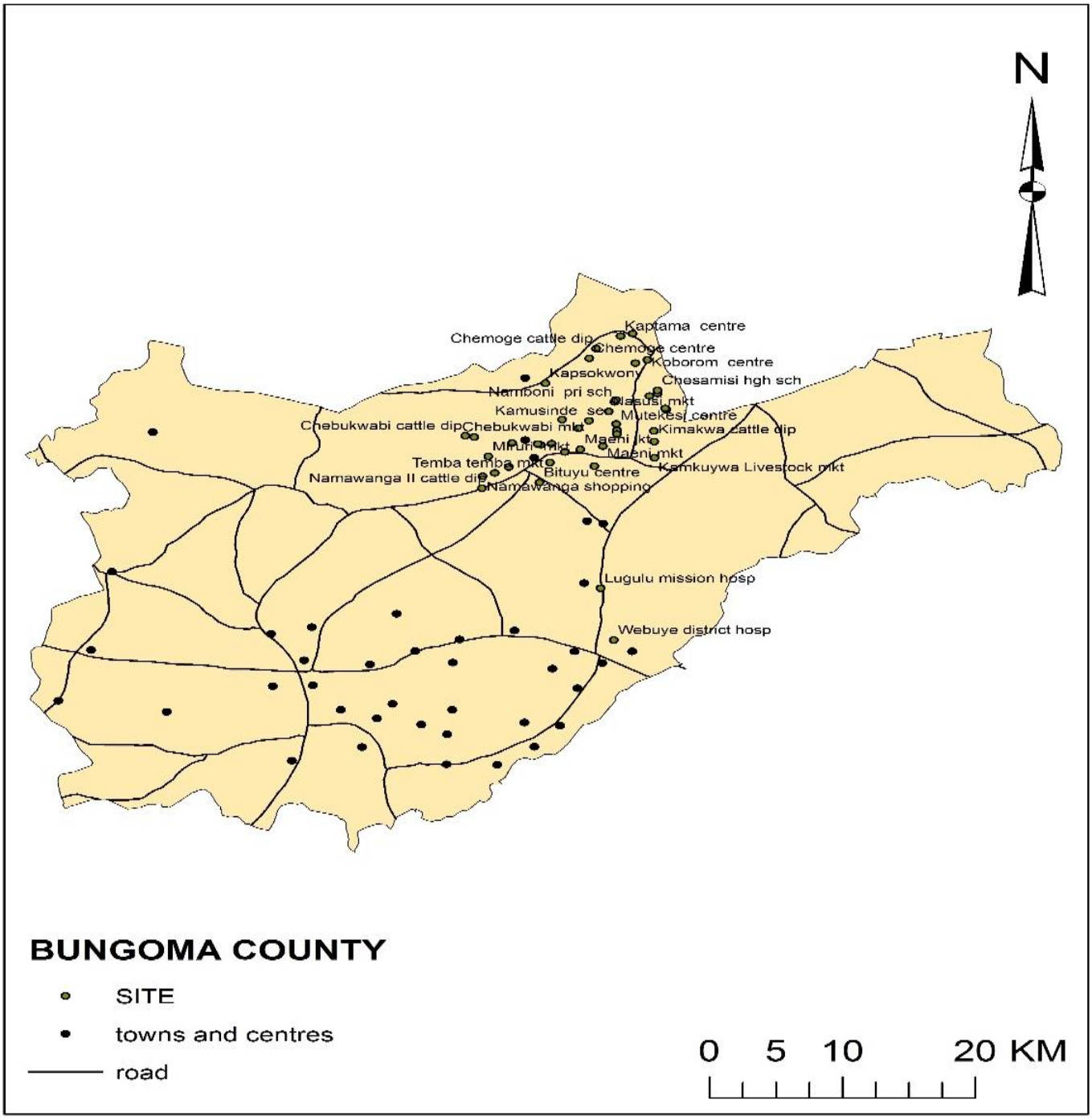

Figure 2
Map of the Study Area Showing where Samples were Collected in Bungoma County, Kenya

IV. FINDINGS & DISCUSSIONS

4.1 The seroprevalence of bovine *leptospirosis* in Kimilili Sub-County Wards and Kaptama Ward Mt.Elgon Sub-County, Bungoma County

Two hundred blood serum samples from cattle were collected in five wards of Maeni, Kamukuywa, Kibingei, Kimilili and Kaptama. In addition, thirty eight (38) blood samples were collected from Kimilili slaughter house, which is the busiest slaughter facility at the study site. The cattle were aged one year and above. Records on the sex of the animals show that 44.5% (N=200) cattle were male and 55.5% (N=200) cattle were female. Then competitive ELISA

test was used to work out the Seroprevalence of *leptospirosis* in Kimilili Sub County wards and Kaptama ward of Mt Elgon Sub-County which constitute the study area.

Antibodies to *leptospirosis* were detected in 16% (N=200) blood sera using ELISA test. Figure 3 shows the sero-prevalence of bovine *leptospirosis* in the study area. The highest Seroprevalence for leptospirosis (21.0%, N=200) was reported in bovine sera obtained at the Kimilili slaughterhouse while the lowest was from Kibingei Ward (10.7%, N=200). The chi square test results on the prevalence is as shown in Table 2 below.

Table 2

Chi Square Test Results

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	24.000[a]	20	.242
Likelihood Ratio	18.729	20	.540
Linear-by-Linear Association	2.255	1	.133
N of Valid Cases	6		

The Chi Square Test on the Seroprevalence figures shown in table 2 above, indicate that there appears to be no significant difference ($p>0.05$) in seroprevalence values in all the wards.

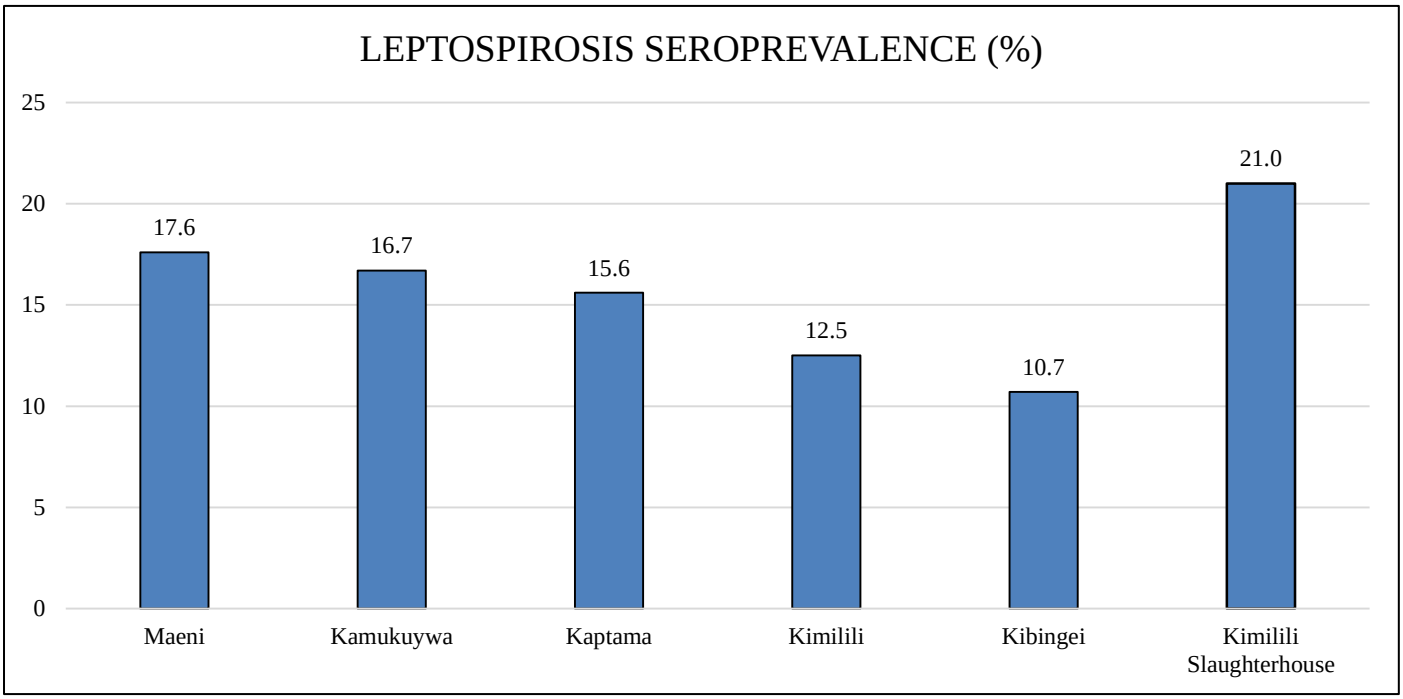

Figure 3

Leptospirosis Seroprevalence in the Wards Kimilili and Mt Elgon Sub-Counties and Kimilili Slaughterhouse in Bungoma County, Kenya

The sex related seroprevalence of leptospirosis in the wards is presented in Table 3. A total number of 89 males and 111 females were tested for leptospirosis. The results show that males had an overall seropositivity of 14.6% while the females recorded 17.1%. The distribution of males testing positive for leptospirosis is shown in Figure 3. The highest percentage of males testing positive is found in Kimilili slaughterhouse (24%), followed by Kamukuywa (16.6%) and Maeni (15.4%) wards respectively.

Table 3

Sex Related Seropositivity of Leptospirosis in Cattle in Bungoma County

Sub-County	Ward	Samples	Sex		Positive
Kimilili	Kibingei	28	M	8	1(12.5%)
			F	20	2(10%)
	Kimilili	32	M	10	1(10%)
			F	22	3(13.6)
	Maeni	34	M	13	2(15.4%)
			F	21	4(19.0%)
	Kamukuywa	36	M	12	2(16.6%)
			F	24	4(16.6%)
	Kimilili slaughterhouse	38	M	29	7(24%)
			F	9	1(11%)
Kaptama	Kaptama	32	M	17	2(11.7%)
			F	15	3(20%)

The chi-square test conducted to check the statistical relationship between sex and leptospirosis seropostivity in cattle returned the results in table 4 below. There was no statistical difference observed between male and female cattle.

Table 4

Relationship between Sex and Leptospirosis Seropostivity in Cattle

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6.333[a]	4	.176
Likelihood Ratio	8.318	4	.081
Linear-by-Linear Association	.112	1	.738
N of Valid Cases	12		

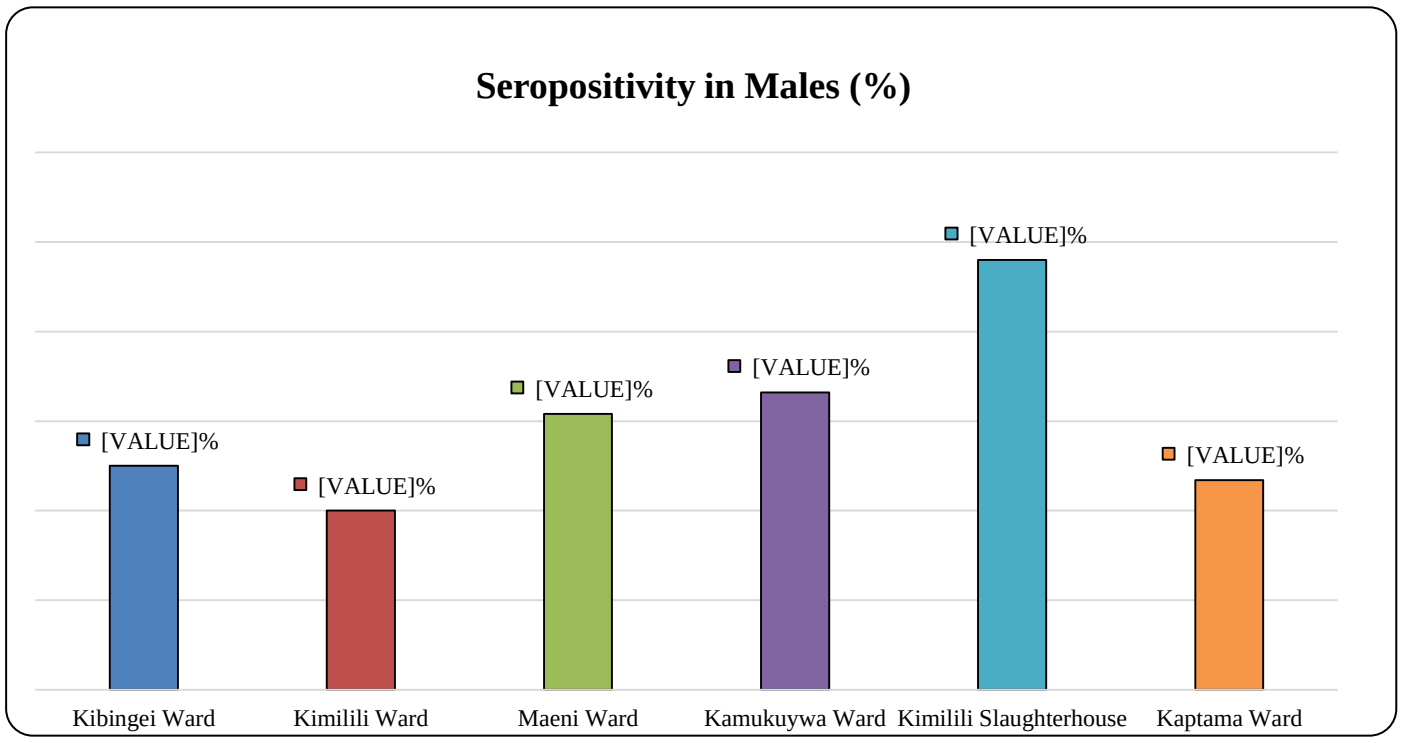

Figure 4

Distribution of Seropositive Male Cattle in Selected Wards in Bungoma County

The distribution of females testing positive for leptospirosis is shown in Figure 4. The highest percentage of females testing positive is found in Kaptama ward (20%), followed by Maeni (19%) and Kamukuywa (16.6%) wards respectively. The lowest percentage of females testing positive for leptospirosis was found in Kibingei ward (10%).

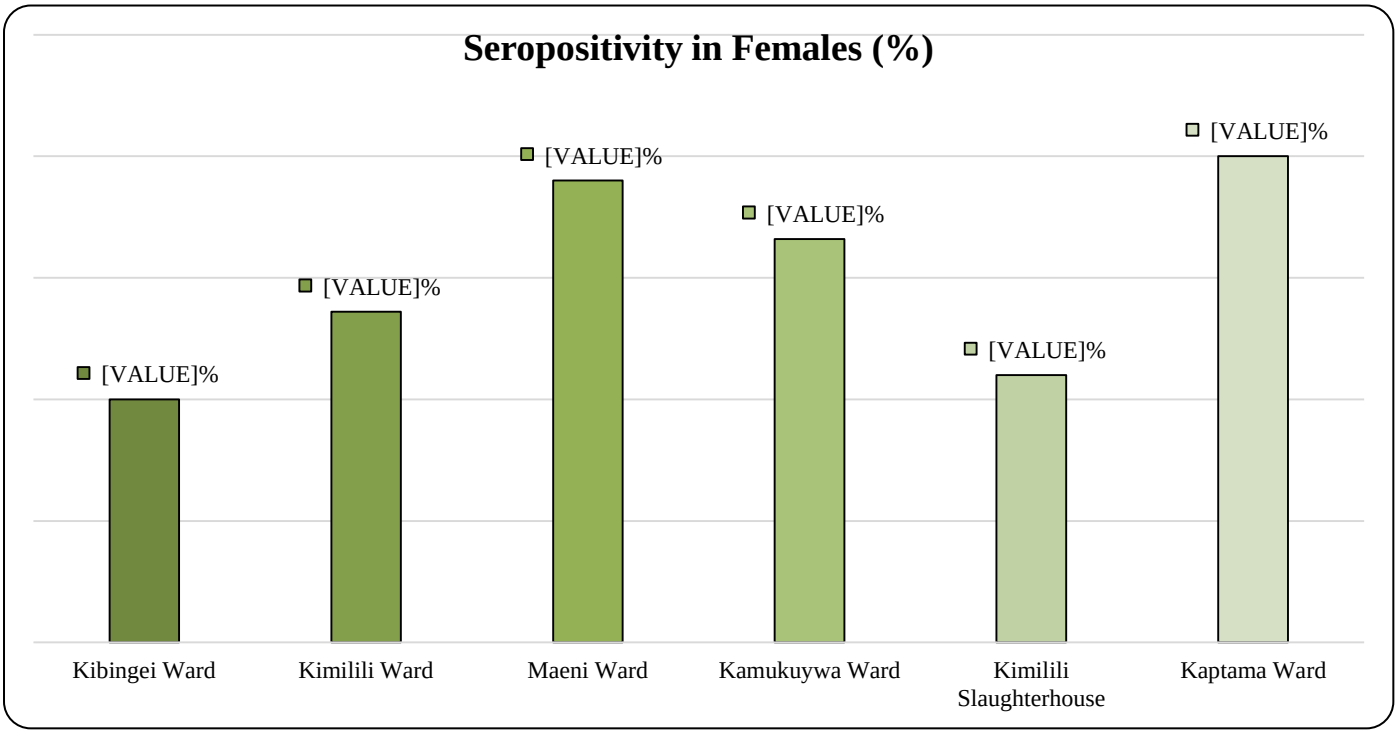

Figure 5
Distribution of Seropositive Female Cattle in Selected Wards of Bungoma County

Since there is no history of vaccination of livestock against *leptospirosis* in Bungoma County, the seropositivity figure obtained from the test is considered a reliable estimate for the cattle exposed to natural infection of *leptospira*. Competitive ELISA test is highly sensitive (96%) and specific (97%) for biological diagnosis of leptospirosis in animal sera for recent as well as past infections (Mythri, 2015). It is of particular value as a serological screening test because of its relative simplicity in comparison with the MAT (Mythri, 2015). However, the ELISA test does not give an indication of the infecting sero-var (Pejvak, 2016).

The high prevalence of *leptospirosis* is consistent with other studies which showed that the prevalence of *leptospirosis* among slaughterhouse workers in Western Kenya is 13.4% (Cook *et al*., 2017). It was also observed that cattle at the slaughterhouse presented the highest sero-prevalence for *leptospirosis*. This is because animals brought to the slaughterhouse originate from different localities ranging from as far as Turkana and West Pokot which are known to have the disease (Macharia, 1989).

The seropositive animals were found in all the five wards under study with the highest seropositivity figures being reported in Maeni (17.6%), followed by Kamukuywa (16.7%), Kaptama (15.6%), Kimilili (12.5%) and Kibingei (10.7%) in that order (Table 1). This demonstrates how widespread the disease is in the study area. The high *leptospirosis* seropositivity in cattle is explained by the fact that there is very high livestock movement in the region because of the very active livestock markets at Kimilili and Kamukuywa livestock sale-yards and a very busy slaughter facility at Kimilili. The high flow of livestock into the area attracts livestock from areas which have high risk for the disease. It was also observed that the highest seropositivity for *leptospirosis* was recorded in cattle from Kimilili slaughterhouse.

Key Informant Interviews confirmed that majority of cattle brought to the slaughterhouse originate from as far as Trans Nzoia, West Pokot and Turkana, Studies and surveillance reports have demonstrated that *leptospirosis* is highly prevalent in the mentioned regions (Macharia, 1989). This study therefore raises concerns about the high risk of exposure to leptospirosis of the slaughterhouse workers in the study area. The need for use of the personal protection equipment is therefore urgent (Cook *et al*, 2017).

A similar study carried out in Kajiado has demonstrated that the sero-prevalence of *leptospirosis* in cattle is 21.8%. The sero-prevalence of *leptospirosis* is higher in Kajiado because of the nomadic lifestyle of the pastoralists where there is widespread movement of livestock and they depend fully on livestock products such as milk, meat and blood for nutrition as compared to Bungoma setup (Nakeel *et al*., 2016). The high sero-prevalence for *leptospirosis* in cattle in Bungoma County demonstrates that there is a high risk of transmission of the disease to humans through contact and consumption of livestock products from their livestock. Through FGD conducted in all the five wards, the severity of *leptospirosis* outbreak as perceived by the respondents was highest in Kamukuywa with a score of 24, followed by Maeni (20), Kaptama (14), Kimilili (12) and Kibingei (5) as shown on Table 1.

Despite the high leptospirosis seropositivity in cattle, there has been no case of leptospirosis recorded in all the livestock in Bungoma County since the year 2000. In addition, surveillance for the disease is not carried out. During FGDs, leptospirosis does not feature as an important disease problem either currently or historically. However, abortion in livestock is reported to be a common problem at the study area (County Director of Veterinary Services [CDVS], 2016). Secondary data from the CDVS reveals that abortion is in cattle is common in both Kimilili and Mt Elgon Sub-counties (CDVS, 2016). Since abortion is one of the manifestations of leptospirosis, it is necessary to carry out further tests to confirm the cause of abortions in cattle and possibly rule out *leptospirosis*. The high seropositivity of leptospirosis shows that leptospirosis is enzootic in the study area and it goes undetected.

This study also reveals that there is a relatively higher prevalence of leptospirosis in females (17.1%) compared to males (14.6%). This finding is consistent with another finding in a study undertaken in pigs in Western Kenya (Ngugi *et al*., 2019). In another study carried out in Vietnam, fattening pigs showed similar findings (Lee *et al*., 2017). This finding is consistent with other studies which suggest that this is due to infection in the female reproductive tract which provides a reservoir for propagation and transmission of the disease (Boqvist et al., 2002). It is also a general practice amongst farmers to keep female animals for a longer time on the farm for milk while most male animals are disposed of earlier for sale leaving only a few for breeding and ploughing. This practice therefore allows females to stay on farm for a longer period of time and therefore increases exposure to leptospirosis (Boqvist *et al*., 2002, Lee *et al*., 2017). The high sero-prevalence of leptospirosis in males at the slaughterhouse on the other hand is attributed to the fact that most of the animals brought for slaughter are males. In addition, they originate from as far as Turkana and West Pokot, areas that are known to have high prevalence of the disease (Macharia, 1989).

Kimilili and Mt Elgon sub-Counties, Bungoma County, Kenya have shown an overall bovine sero-prevalence to be is 16%. In addition, positive cases of leptospirosis in cattle were encountered in all the five wards of the two sub-counties and Kimilili slaughterhouse. The highest sero-prevalence figures were recorded from cattle at the slaughterhouse (21.0%) followed by Maeni Ward (17.6%), Kamukuywa Ward (16.7%), Kaptama Ward (15.6%), Kimilili Ward (12.5%) and Kibingei Ward (10.7%) in that order.

V. CONCLUSIONS & RECOMMENDATIONS

5.1. Conclusion

This study demonstrates that leptospirosis is enzootic and there is a high risk of contracting the disease at the study area. The disease therefore presents a serious public health problem in both humans and livestock.

5.2. Recommendations

Based on the study, it is recommended that concerted efforts in combating leptospirosis be initiated through public education to sensitize communities about the disease. The relevant authorities should also initiate surveillance coupled with laboratory diagnosis for early detection and control of the disease. Slaughterhouse workers should also take necessary precautions to avoid the risk of contracting the disease.

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