

1 ***Leptospira* spp. seroprevalence in humans involved in a cross-sectional study in Garissa and Tana**  
2 **River Counties, Kenya**

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17

18 **Abstract**

19 **Introduction:** Leptospirosis is a neglected bacterial zoonotic infection caused by spirochetes of  
20 *Leptospira* genus. Humans get infected through direct or indirect contact with urine of infected animals  
21 or environment. It accounts for more than 300,000 severe cases annually worldwide with case fatality  
22 rates of over 30%. Costs of diagnosis and treatment for human and animals, disruption of international  
23 trade of animals and products, reduced productivity and reproductivity in animals constitute economic  
24 importance. In Kenya, leptospirosis burden is significant but under-diagnosis and under-reporting affects  
25 the awareness of the disease. This study aimed to determine and compare the sero-prevalence and factors  
26 associated with *Leptospira* spp. in the two counties.

27 **Methods:** We conducted a cross-sectional study that involved apparently healthy people of at least 5 years  
28 of age in randomly selected households in Garissa and Tana River Counties. Blood samples were collected  
29 and tested for *Leptospira* spp antibodies using IgM ELISA. Standardized structured questionnaires were  
30 administered to collect socio-demographic and exposure information. We calculated frequencies and  
31 proportions for categorical variables and odds ratios (OR) and 95% confidence interval (CI) to evaluate  
32 association between sero-positivity and exposure factors. We used Wilcoxon test to evaluate statistical  
33 difference in sero-positivity for continuous variables and calculated test statistic (H) and p-value.

34 **Results:** A total of 952 subjects were recruited into the study – these included 482 persons from Garissa  
35 and 470 from Tana River. The overall sero-prevalence was 26% [(244/952); (CI: 23% to 29%)]. Garissa

36 County had significantly higher *Leptospira* spp. seroprevalence (31%, n = 147; CI: 27% to 35%) compared  
37 to Tana River County (21 %, n = 97; CI: 17% to 25%). Being a female (OR=1.6, CI: 1.2-2.2) and engaging  
38 in pastoralism (OR=2.7, CI: 1.8-3.9) were significantly associated with higher odds of *Leptospira* spp.  
39 seropositivity compared to being a male or working in irrigated areas. The mean altitude of residence of  
40 sero-positive patients was 73m ± 21 SD (standard deviation) above sea level and that for sero-negative  
41 was 80m ± 22 SD (H=35, p-value = 0.00).

42 **Conclusion:** This study determined the seroprevalence and risk factors for *Leptospira* spp. exposure in  
43 Garissa and Tana River Counties, Kenya. Females in pastoral communities experience high burden of the  
44 disease. Enhanced surveillance in humans and animals and further research is required to understand the  
45 complex and multifactorial drivers of leptospirosis transmission in the two Counties.

46

## 47 **Introduction**

48 Leptospirosis is a neglected bacterial zoonotic infection caused by spirochetes of the genus  
49 *Leptospira*. Humans mainly get infected through direct or indirect contact with urine of infected  
50 animals or contaminated environment [1]. *Leptospira* spp. seroprevalence vary significantly  
51 between and within countries, based on environmental settings, behavioral risk factors, and socio-  
52 demographics. The majority of the morbidities and mortalities occurs in regions which have large  
53 subsistence farming, pastoral populations and where the disease is a veterinary health problem  
54 [2]. Its impacts are associated with reduced livestock production, high costs of detection and  
55 treatment in human and animals, and disruption of international trade of animals and its products.

56

57 It is estimated that 7 to 10 million people are infected by leptospirosis globally with about 1.03  
58 million cases and 58,900 deaths occurring due to leptospirosis annually. This translates to  
59 approximately 2.90 million Disability Adjusted Life Years [3]. A large proportion of cases (48%)  
60 and deaths (42%) occur in adult males within age of 20–49 years [2]. In Africa, *Leptospira*  
61 incidence has been estimated to be 95.5 cases per 100,000 and prevalence ranges from 2.3% to  
62 19.8% [4]. A few studies have been done in Kenya on the disease; available data show that in  
63 1987, 7.4% of 353 healthy people in Nyanza region and 16.9% of 130 in the coastal region had  
64 *Leptospira* antibodies [5]. Later in 2012, a cross-sectional survey that involved slaughterhouse  
65 workers in Busia, Kenya reported a prevalence of 13.4% [6].

66

67 Although leptospirosis is known to be one of the high consequence zoonotic disease worldwide,  
68 there is limited knowledge on its burden, spatial distribution, and relative importance of the

69 disease in Kenya. The disease has been listed among the top 20 priority diseases in the country  
70 [7], but there is inadequate knowledge on its distribution, risk factors and the most vulnerable  
71 groups in the country. Its surveillance is inadequate, and there have not been any investments on  
72 community sensitization campaigns to improve its detection and reporting. The disease is under-  
73 diagnosed and under-reported due to multiple challenges including nonspecific clinical symptoms  
74 [8–12]. It is one of the neglected diseases [13,14], and in resource-constrained countries (e.g.  
75 Kenya) that has multiple diseases and socio-economic challenges to confront, quantification of  
76 leptospirosis burden would guide re-evaluation of disease priority lists [11,15]. This study  
77 determined *Leptospira* spp. seroprevalence and risk factors in selected areas in Garissa and Tana  
78 River Counties, eastern Kenya.

79

## 80 **Methods**

81

### 82 **Study site**

83 The study was conducted in Bura and Hola, Tana River County, and Ijara and Sangailu in Garissa  
84 County. These sites have been described earlier [16]. Briefly, Bura site was in an irrigation and  
85 settlement scheme that covered 2,100 hectares with an approximate population of more than 2,000  
86 households spread out in 10 villages. Hola study site was in a neighbouring irrigation and settlement  
87 scheme that covered 1,011 hectares with 700 farming households in 6 villages. They received  
88 approximately 460mm per year which peak in October–December. Their average daily temperatures  
89 ranged between 32 - 37°C. Ijara and Sangailu fell under Ijara sub-County which borders Lamu County  
90 and Boni forest to the East and Tana River County to the West. This is an arid/semi-arid area where  
91 pastoralism is practised. Their annual rainfall ranged between 750mm – 1000 mm while the mean  
92 temperature ranged between 15°C - 38°C. Sampling was done from December 2013 to February 2014.

93

### 94 **Study design**

95 The study used a cross-sectional study design with households as the primary sampling unit, and  
96 subjects in households as secondary units. In Bura and Hola, lists of households that were used as the  
97 sampling frame were obtained from the local irrigation schemes. In Ijara and Sangailu, lists of  
98 households were developed with the help of the village headmen.

99

100 The number of people/households to sample was determined using a sample size for estimating a  
101 population proportion. A minimum sample size of 845 was estimated assuming that *a priori* *Leptospira*

102 spp. seroprevalence was 50%, level of confidence was 95%, reliability 5% and that the correlation  
103 coefficient,  $\rho$ , of seropositivity outcome at a household level was 0.3. It was further assumed that up  
104 to five people per household,  $m$ , would be sampled.

105

## 106 **Sampling methods**

107

### 108 **Study Population**

109 The sample size was uniformly distributed to all the study areas in order to have 211 subjects or 42  
110 households per site. These households were randomly identified from the sampling frames using  
111 computer generated random numbers. Within each household, the household head was asked to identify  
112 up to 5 people for sampling. These had to be participants that were healthy above the age of 5 years at  
113 the time of study. Children below the age of 5 years were not included in the study.

114

### 115 **Data collection**

116 We administered a standardized questionnaire to the study participants to collect socio-  
117 demographic, household and area level data that would be used to determine risk factors for  
118 *Leptospira* spp. exposure. Variables collected included age, sex, occupation, education status,  
119 total number of people in a household, source of water, geographical coordinates and altitude and  
120 the main land use activity per sampling site. These data were collected using the Open Data Kit  
121 (ODK) application in android-enabled smartphones. The data were posted at the end of each day  
122 to an on-line server at the International Livestock Research Institute (ILRI).

123

124 Blood samples were then collected from up to five people in a household. The household was  
125 given the authority to identify a household member who would be sampled provided they were  
126 more than 5 years. Recruited subjects were seated comfortably for blood collection. Up to 10ml  
127 venous blood was drawn from the left median cubital vein after disinfecting the injection site  
128 using 70% isopropyl alcohol. A tourniquet was placed about 3-4 inches above the venipuncture  
129 site. Sterile butterfly needles and vacutainer tubes were used to draw blood which was collected  
130 into non heparinized tubes. Blood samples were transported in a cool box to a field laboratory  
131 where they were centrifuged at 3000xg for 10 minutes. Serum harvested was aliquoted to 2ml  
132 cryotubes in duplicates and kept in dry ice until transported to ILRI Nairobi where it was preserved  
133 at -80°C until analyzed.

134

135 The samples were screened for anti-*Leptospira* spp. IgM using Panbio® *Leptospira* IgM ELISA. The  
136 kit is known to detect infections caused by a wide range of *L. interrogans* serovars including: hardjo,  
137 pomona, copenhageni, australis, madanesis, kremastos, nokolaevo, celledoni, canicola, grippotyphosa,  
138 szwajizak, djasiman and tarassov. A case was defined as a positive human serum sample for *Leptospira*  
139 antibodies on Panbio® *Leptospira* IgM ELISA

140

#### 141 **Data analysis**

142 Seropositivity was assumed to represent the odds of *Leptospira* exposure in people. Data posted to an  
143 online server at ILRI were transferred to a relational database designed using MS Access. These were  
144 merged with the laboratory results, cleaned and analysed using descriptive and analytical models.

145

146 Descriptive analyses included the determination of the number of subjects, households, and villages  
147 sampled. The distribution of each variable was also analysed. *Leptospira* seropositivity was considered  
148 as the dependent variable, while gender, age, occupation (pastoralist, farmer, student and other), source  
149 of water (borehole, canal, dam and other), livestock ownership (yes/no), family size, land use (pastoral,  
150 irrigation, riverine), location (Sangailu, Ijara, Bura and Hola) and altitude were independent variables.  
151 Age was initially analysed as a continuous variable, but it was later transformed to categorical variable  
152 with three levels – as <20, 20 – 40 and >40 years – when it failed to meet the linearity assumption.  
153 Occupations grouped under the other category included businesspersons, employed, driver, village  
154 chief and a nurse, among others.

155

156 Bivariate analysis involving the outcome and all the categorical variables was performed and  
157 *Leptospira* spp. seroprevalences and their 95% confidence intervals were calculated at the various  
158 levels of these variables. Chi-square tests were also used to assess crude associations between each  
159 factor and the dependent variable (at  $\alpha = 0.05$ ). The variation in *Leptospira* spp. with continuous  
160 variables, including altitude at the sampling site and the size of the household were analysed using T  
161 Test. These were succeeded by univariable regression models involving each of the variables using a  
162 crude logistic regression model. Variables that were significant or returned a  $p < 0.2$  were included in  
163 multivariable modelling.

164

165 Multivariable analysis used a mixed effects logistic regression model, implemented using *melogit*  
166 command in STATA version 14, to account for clustering of data at the household and village levels.  
167 A mixture of backward and forward variable selection technique was used involving all the variables

168 that met the criteria specified under the univariable analysis described above. Two random effects  
169 variables – village ID and household ID -- were fitted, and their significance assessed using the  
170 likelihood ratio test. Fixed and random effects variables were retained in the model if their likelihood  
171 ratio tests were significant, assuming a type II error,  $\alpha$ , of 0.05. The residual intra-cluster correlation  
172 coefficient was estimated using the command *etstat icc* after running the final model. Residual analysis  
173 using standardised residuals was conducted to identify outliers.

174

## 175 **Ethical considerations**

176 We sought ethical approval from the African Medical and Research Foundation (AMREF) Ethics and  
177 Science Review Committee (ESRC). An approval number provided was AMREF-ESRC P65/2013.  
178 Informed consent was sought from participants and assent for children below 18 years. The participants  
179 were assured of confidentiality and anonymity. Benefits and/or risks associated with the study were  
180 explained to the participants who had freedom to withdraw from the study at any time. The researcher  
181 ensured safety of data collected through access restrictions by use of passwords and storage in lockable  
182 lockers.

183

## 184 **Results**

185

### 186 Descriptive statistics

187

188 A total of 952 subjects were recruited into the study. Their mean age was 29.9 (95% confidence interval:  
189 28.7 – 31.2) years. Most of them were female (59.1%, n = 562). The subjects came from 347 households  
190 distributed in 40 villages in Tana River and Garissa counties. The number of subjects sampled per  
191 household and village ranged between 1 - 5 and 3-56, respectively. Households however had more  
192 members with the mean household size being 8.6 (95% CI: 8.4 – 8.9). The study area had a mean elevation  
193 of 78.7 (SE = 0.69) m above sea level A detailed analysis of the characteristics of the subjects is given in  
194 Table 1.

195

### 196 *Leptospira* spp. seroprevalence

197 The overall *Leptospira* spp. seroprevalence was 25.6% (95% CI: 22.9 – 28.5). Table 1 shows the variation  
198 in *Leptospira* spp. seroprevalence by categorical variables considered in the study. All the subject-level  
199 variables (sex, occupation and age category) had significant effects while the household categorical

200 variables – source of water and ownership of livestock – were not. Area-level variables (land cover and  
201 the identify of an area) were also significant.

202

203 Two other continuous variables – the size of a household and altitude –were also significantly associated  
204 with *Leptospira* spp. seroprevalence. Household sizes for the seropositive subjects were significantly  
205 larger (mean of 9.2 persons; 95% CI: 8.7 – 9.7) than the those for seronegative ones (8.4; 8.1 – 8.7).  
206 Similarly, the mean elevation for the seropositive subjects (73.0m; 95% CI: 70.3 – 75.7m) was  
207 significantly lower than that for seronegative subjects (80.7m; 95% CI: 79.2 – 82.3m).

208

209 Table 1. Variation in *Leptospira* spp. seroprevalence by categorical variables defined at the subject,  
 210 household and area levels  
 211

Variable	Levels	n	Seroprevalence		
			%	95% CI	$p > \chi^2$
Sex	Male	389	20.57	16.66 – 24.93	0.00
	Female	562	29.18	25.45 – 33.13	
Occupation	Pastoralist	255	33.33	27.57 – 39.48	0.00
	Farmer	163	14.11	9.16 – 20.42	
	Student	121	14.88	9.06 – 22.49	
	Other <sup>1</sup>	68	23.53	14.09 – 35.38	
Age	≤20	355	19.15	15.19 – 23.64	0.00
	20 - 40	307	33.22	27.98 – 38.80	
	>40	289	25.61	20.67 – 31.05	
Source of water	Borehole	128	21.09	14.38 – 29.19	0.54
	Canal	223	19.73	14.71 – 25.57	
	Dam	336	24.70	20.18 – 29.67	
	Other	29	20.70	7.99 – 39.72	
Livestock ownership	Yes	687	22.42	19.35 – 25.72	0.83
	No	29	20.69	7.99 – 39.72	
Land use	Irrigation	252	15.08	10.90 – 20.11	0.00
	Pastoral	625	30.78	27.12 – 34.50	
	Riverine	71	19.72	11.22 – 30.87	
Location	Ijara	141	40.25	33.94 – 46.81	0.00
	Sangailu	194	21.13	16.20 – 26.78	
	Tana North	244	17.29	13.15 – 22.10	
	Tana South	129	26.29	19.93 – 33.46	

212

213 Univariable and multivariable analyses

214

215 All the variables identified above were fitted in a logit model. All the variables except livestock ownership  
 216 and source of water were significant. These two insignificant variables were therefore not considered  
 217 further in the analysis.

218

219 The final multivariable model fitted to the data is given in Table 2. Three fixed effects (gender, occupation  
 220 and location) and one random effect (household) variables met the criterion for inclusion in the model.  
 221 Age and occupation as well as location and altitude could not be kept in the model at the same time; the

222 presence of occupation in the model made age to be insignificant. Similarly, location rendered altitude  
 223 insignificant. In both cases, predictors that provided the greatest log-likelihood estimates were preferred.

224

225 The results given in Table 2 suggest that being a female was associated with higher odds of exposure to  
 226 the pathogen than being a male. Similarly, pastoralists had significantly greater odds of exposure than  
 227 farmers and students. Ijara had significantly higher *Leptospira* spp. seroprevalence compared to all the  
 228 other three sites (Bura, Sangailu and Hola).

229

230 Household ID, fitted as a random effects variable, was significant in the model ( $\chi^2 = 8.24$ ,  $p = 0.00$ ). The  
 231 residual intra-class correlation coefficient associated with this effect was 0.27 (95% CI: 0.12 – 0.51).

232

233 Table 2. Outputs of a random effects logistic regression model used to determine factors that affect the  
 234 seroprevalence of *Leptospira* spp. in humans in Tana River and Garissa counties, Kenya

Variable	Level	Odds Ratio			Z	P>Z
		Mean	SE	95% CI		
<i>Fixed effects</i>						
Sex	Male	0.58	0.15	0.35 - 0.97	-2.08	0.04
	Female	1.00				
Occupation	Farmer	0.33	0.13	0.16 - 0.71	-2.86	0.00
	Other	0.67	0.29	0.28 - 1.57	-0.93	0.35
	Student	0.36	0.14	0.17 - 0.77	-2.63	0.01
	Pastoralist	1.00				
Location	Ijara	3.30	1.57	1.30 - 8.37	2.52	0.01
	Sangailu	0.75	0.33	0.32 - 1.76	-0.65	0.51
	Hola	1.42	0.51	0.70 - 2.87	0.98	0.33
	Bura	1.00				
Constant		0.40	0.14	0.20 - 0.82	-2.53	0.01
<i>Random effect</i>						
	Household ID	1.22	0.65	0.43 - 3.44		

235 Log Likelihood = -303.59; number of records = 607; number of groups = 302

236

## 237 Discussion

238 This study investigated the seroprevalence of *Leptospira* spp. in people who lived in pastoral (Garissa)  
 239 and agropastoral (Tana River) counties, eastern Kenya. Leptospirosis is a neglected zoonotic disease  
 240 whose distribution in the country is poorly known. It has also not been included in the list of diseases  
 241 that should be screened for while investigating causes of febrile illnesses in humans. Our findings –  
 242 which suggest a high seroprevalence of the disease in the study areas, more so in pastoral areas – show  
 243 that awareness on the disease and its risk factors should be enhanced.

244

245 Both study regions used (Garissa and Tana River counties) lie on the either side of Tana River, a major  
246 river in Kenya that emanates from the slopes of Mt Kenya and terminates in the Indian Ocean. Their  
247 climatic conditions and vegetation cover provide good environmental conditions that support a wide  
248 range of wild animals. Livestock and wild animals act as reservoirs of *Leptospira* [16–18] and it is  
249 likely that silent transmissions of *Leptospira* spp. occur between livestock and wildlife since they share  
250 common grazing and watering resources.

251

252 One of the main but unexpected finding of the study was that *Leptospira* seroprevalence was  
253 significantly higher in the pastoral (Garissa) areas compared to agropastoral or irrigated areas in Tana  
254 River County. Outbreaks of leptospirosis is often precipitated by flooding and it was thought that  
255 irrigated areas would have higher seroprevalence. One possible explanation for this finding is that most  
256 of the people sampled in Garissa, especially those that lived miles away from River Tana, obtained  
257 water for domestic use from open water pans that had been built to trap rain water. These pans however  
258 served as common watering points for livestock and wildlife. They waded into the waters while drinking;  
259 they often urinate in and around these pans during or after taking a drink. People sampled in Tana River  
260 could access flowing water supplied to them via irrigation canals.

261

262 Water fetched from water pans in the pastoral areas could be boiled before use to reduce the risk of  
263 exposure to *Leptospira* spp. However, there would still be multiple opportunities for contact, especially  
264 for women and children who play a major role in fetching the water, or for the young men who took  
265 animals for watering in these pans. Our results showed that female gender, engaging in livestock related  
266 activities were associated with *Leptospira* seropositivity. We observed that age group of 21-30 were  
267 more likely to be seropositive. Higher infection rates in this age group corroborates findings in the  
268 majority of leptospirosis studies around the world, given the multiple but high risk responsibilities that  
269 people in this age group are assigned to (e.g. fetching water, taking care of animals, increased outdoor  
270 activities and recreational exposures, e.g. swimming). Seropositivity rates are lower in older age groups  
271 as risky exposure behaviours reduce [2,15].

272

273 Other gender-related activities that would force women in pastoral communities to come into direct  
274 contact with potentially contaminated water include washing, cleaning utensils, and cleaning their  
275 houses. This further shows that unlike other diseases where men often suffer higher risk of exposure  
276 given their outdoor occupations, *Leptospira* exposure may be more important in women than men in

277 pastoral areas. Similar findings have been made in a study that used National health survey data in  
278 Chile [24].

279

280 Of importance to future *Leptospira* cross sectional studies in the region is the documentation of  
281 significant variation in the number of cases between households. An intra-cluster correlation  
282 coefficient of 0.27 (95% CI: 0.12 – 0.51) was estimated from the study. This demonstrates that  
283 households had significant differences on the extent to which they were exposed to the hazard. It  
284 therefore demonstrates that there is room to improve the management of *Leptospira* exposure in these  
285 communities for instance through behaviour change communication. The study had a few limitations  
286 though. It measured antibodies for *Leptospira* to identify evidence of prior infection. However, many  
287 *Leptospira* infections are subclinical. The development of clinical disease is dependent on multiple  
288 factors including pathogenicity of the serovars, individual's immune status, comorbidities, and age.  
289 This may cause unnecessary alarm to the public health in terms of the high prevalence found.

290

291 We conclude that *Leptospira* is high in Garissa and Tana River counties. Females in pastoral areas  
292 had higher odds of exposure compared to males. Leptospirosis has not received sufficient attention  
293 despite the magnitude of the public health threat it poses and its negative economic impact on  
294 development and livelihoods in rural communities in low income countries. It is therefore  
295 important to gather data on the burden of leptospirosis to enable decision-makers to develop policy  
296 aimed at mitigating the effects based on reliable scientific evidence.

297

298 We recommend the inclusion of *Leptospira* surveillance in the disease surveillance and response  
299 (DSRU) platform being implemented by the Ministry of Health to determine the extent of the problem  
300 and its occurrence patterns. A 'One Health' approach to leptospirosis research and control should also  
301 be promoted to improve understanding of the epidemiology of the disease.

302

303

### 304 **Acknowledgements**

305 We acknowledge the International Livestock Research Institute, University of Nairobi, Ministry of Health  
306 (Division of Disease Surveillance and response), Kenya Medical Research Institute, Kenyatta National  
307 Hospital, Director of Veterinary Services-Kenya and Kenya Field Epidemiology and Laboratory Training  
308 Program for their funding and/or collaboration in this study.

309

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Figure 1: Flow chart showing the prevalence of Leptospirosis in Garissa and Tana River Counties, Kenya, December 2013-February 2015,