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The Power of Multiple Linear Regression Models to Predict Apparent Density of *Glossina fuscipes fuscipes* (Diptera: Glossinidae): A Case Study of Kajo-Keji County, Central Equatoria State, South Sudan

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ABSTRACT

Glossina fuscipes fuscipes continue to be the primary tsetse vectors of *Trypanosoma brucei gambiense*, the cause of Human African Trypanosomiasis (HAT), in South Sudan, where the HAT Control Strategy does not include a vector control component. Priority regions for vector control can be determined using data on fly apparent density/ trap/day. Insecurity and logistic problem makes it impossible for vector control activities to be carried out, therefore, there is a need for an alternative method to assess vector population without having physical presence in fields. What is needed under these circumstances are the environmental parameters that influence vector population density in the study area. Such variables are always available in meteorological stations in the Country. The study aims at providing information on *Glossina fuscipes fuscipes* apparent density/trap/day in Kajo-Keji County by employing Multiple Linear Regression Models with input from environmental variables (Atmospheric temperature, precipitation, relative humidity and wind speed). Tsetse field surveys were conducted along 8 streams in the study area from January to December 2012. To estimate fly apparent density/trap/day as a function of probable factors for tsetse fly catches, twelve linear regression models were created. The paired samples T-test in SPSS was used to investigate the

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discrepancy between the fly apparent densities produced by the models and the actual densities from the survey. The top and lower limits of the model agreements were 5.97 and -11.65, respectively, and the prediction values of the models demonstrated the monthly trends of *G. fuscipes fuscipes* abundance. The model appears fit for the data and prediction of the fly apparent density from the various predictors ($F(4,11) = 14.321, P < 0.02$). The densities predicted by the models did not vary statistically ($df=11; P = 0.69$) from the actual ones. This study could contribute to provide information on the peaks of the vector abundance that may guide strategic plans for tsetse and HAT control programmes in South Sudan. Multiple Linear Regression Models are robust and flexible and could find applications in the various aspects of tsetse studies and provide useful information for tsetse and trypanosomiasis control programmes in South Sudan.

Keywords: *Glossina fuscipes fuscipes*; apparent density; multiple linear regression models; environmental factors.

1. INTRODUCTION

Tsetse fly (*Glossina* sp.) is the primary vector for trypanosomes, the protozoal parasites that cause trypanosomiasis in humans and animals, and these vectors are classified into three subgroups: riverine subgroup known as the “palpalis”, the savannah subgroup called the “morsitans subgroup” and forest-dwelling tsetse known as the “fusca” [1]. The most significant biological carriers of Human African Trypanosomiasis (HAT), accounting for about 90% of all illness cases in Africa, are *Glossina fuscipes* [2]. Patches of *G. fuscipes fuscipes* have also been found on the margins of Lake Victoria in Tanzania [3], as well as in Southwestern Ethiopia and South Sudan [4]. In South Sudan/ Sudan, *G. f. fuscipes* are the main vectors of *Trypanosoma brucei gambiense*, though *G. tachinoides*, *G. pallidipes*, and *G. morsitans* have also been found in the Greater Equatoria Region [5,6]. Several studies have shown that *G. f. fuscipes*, a riverine species of the Palpalis group, prefer dense vegetation on river banks as habitats with conducive conditions of humidity, warmth and light prevail [7]. River banks provide source of blood supply to tsetse from the dwellers during water collection.

Knowledge of how environmental factors and their drivers influence Tsetse population density May provide vital information on tsetse (*G. f. fuscipes*) population and such information may be crucial for tsetse control and intervention programmes. Effects of these environmental factors/predictors on the monthly apparent density of *G.f.fuscipes* can be best quantified using regression models. Regression is a statistical tool used to quantify the association between an outcome measure and predictor variables. This approach has been used in the predictive mapping of various vectors and associated vector-borne diseases, including malaria and Rift Valley fever (RVF), with broad applications in environmental disease risk [7].

Multiple regression models are often used in many study areas using simple assumptions [8]. This model comprises both independent and dependent variables, and is easily verified, based on three viewpoints. The first of which is the correctness of the values predicted by the model. The second is the multicollinearity between independent factors, and the third is whether the errors in the model have normality or not. Several studies have been carried out using Multiple Linear Regression Model (MLRM) for forecasting Bluetongue disease outbreak in sheep in India [9] and adult female *Aedes aegypti* in Saudi Arabia [10]. Similarly, the model has been applied to study the potential impacts of climate change on stable flies [11] and to predict mosquito abundance and habitats in USA [12].

Tsetse flies are vectors of human and animal trypanosomiasis in sub-Saharan Africa and are the targets of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) [13]. It is evident that HAT remains one of the important Tropical Neglected Diseases (TNDs) threatening human health in sub-Saharan Africa [14,15]. Studies in Uganda and the Democratic Republic of Congo have shown that HAT can impact the functioning of households with the consequences of increased poverty; decline in agricultural activities often leading to famine or lack of basic food and nutrition security; disruption of children's education and; generally, reversal of role in obligations, which are more often than not enhance women's and children's burdens [16]. As a result of Glossina-borne parasite that causes HAT, it has been found that approximately 1.6 million Disability Adjusted Life Years (DALYs) is due to HAT and considered second among all vector-borne diseases in Africa for mortality and fourth for related disability [17]. Human African Trypanosomiasis has seriously impacted populations with greater social, cultural, and economic vulnerabilities [18] settlements and economic developments in most African countries, particularly those south of the Sahara Desert where it is transmitted mainly by tsetse flies [19].

In South Sudan, Gambian HAT control activities rely mostly on case detection and treatment of the detected cases. World Health Organization (WHO) has targeted elimination of HAT as public health problems by 2020 [20]. South Sudan HAT control programme does not focus on Vector Control as a component of HAT control and intervention. However, Tsetse Vector Control initiated by PATTEC in South Sudan since 2009 [21]. Yet, the programme has never been well implemented mainly due to insecurity problems in some foci of HAT and/or logistics constraints. Case detection strategy for HAT control programme hardly covers more than 75% of the population and an alternative method is to eliminate the tsetse fly which transmits the parasite causing the disease [20]. Therefore, vector control remains an important component of HAT control and elimination programme. Vector control has the advantage of completely interrupting HAT transmission although it is too expensive and difficult to carry out in resource-poor settings [22]. The need for tsetse vector control component in HAT control programme may speed up HAT eradication as advocated by PATTEC. Implementing tsetse vector control needs enough resources among many others. Therefore, financial, logistics and technical constraints could hinder tsetse vector control activities. These problems of insufficient resource allocation for vector

control programmes may be solved by prioritizing certain areas where the vector control activities have achieved greater impacts. However, priority areas for vector control programme deployment is based on fly apparent densities. So far, there has no information about the apparent densities of *G. f. fuscipes* in South Sudan. Therefore, up-to-date information is needed for the numbers of flies caught/trap/day as a function of environmental predictors for decision-making processes and improved planning for tsetse control interventions. The Multiple Linear Regression Model (MLRM) method may be applied to give an insight into the fly density once all the necessary environmental predictors of the sites or the areas are obtained either from the country's meteorological station or from any other reliable sources. In situations where vector control intervention is infeasible, knowledge on the fly apparent densities remains needed. Because human tsetse contacts play a role for *T. b. gambiense* transmission, the level of human exposure to tsetse flies in the areas of human tsetse contacts can be reduced [20] and this will prevent at least HAT transmission to a proportion of population in those areas with tsetse infestation. This paper discusses how the apparent density of *G. f. fuscipes* is predicted from environmental predictors using the MLRM. These models could be applied to other studies that predict the effects of climate change on *G.f. fuscipes* infestation rates, feeding behavior and tsetse parasite interaction.

2. METHODOLOGY

2.1 Description of the Study Area

Kajo-Keji County (KKC) lies between latitudes 3.67203- 4.13238 °N and longitudes 31.1004 -31.8172 °E. The County covers an area of approximately 113,000 km² bordering the neighboring Uganda in the South, Yei River County in the West, Juba County in the North and the River Nile in the East. KKC is an area of the tropical rainforest with moderate soil fertility and the climate is marked by minimal variations in seasonal temperatures. The annual rainfall ranges between 1,200 and 2,000 mm for about 8 months from March to October.

2.2 Entomological Survey

2.2.1 Sampling and sample size

Tsetse field surveys were carried out in the study area from January 2012 to December 2012. Sampling of flies was conducted for five consecutive days in each month as from 8:00 a.m. to 4:00 p.m. for 12 months during wet and dry seasons. Tsetse samples were taken from eight streams which include Lorini, Kungupiri and Sanga in Lire Payam; Tenderi in Kangapo I Payam; Kibo, Lowiyu and Nyawa in Kangapo II Payam as well as Koyibo in Liwolo Payam. KKC is endowed with a number of streams. The banks of these streams are inhabited with various types of vegetation covers, trees and tsetse flies. The habitats on the bank of each stream are classified into single, double and peri-domesticated forest galleries based on their vegetation covers, trees and other ecological attributes [23]. The sample size of tsetse was determined by 95% confidence interval at a desired level of 5% [24] and the stratified random sampling method

was used for monitoring the prevalence of tsetse and assessing species' diversity and distribution. Unbaited biconical traps were deployed in seven different sites along the banks of the eight streams [25]. These traps were deployed 150 m apart and 5 m distant from the streams [26]. The deployment of the traps occurred once every week during both the wet and the dry seasons. Captured flies were collected every 24 h, counted, and stored in cool boxes.

2.2.2 Calculation of tsetse apparent density

The fly apparent density/trap/day (AD) was calculated as described by Dede et al. [27] as follows:

$$AD = \frac{\text{Number of flies caught/trap}}{\text{day}}$$

2.3 Measurement of Environmental Variables

Data for the monthly rainfall, temperature and relative humidity for the year 2012 were obtained from Juba National Meteorological Department. Similarly, the daily wind speed for 12 months was downloaded from the website www.yr.no. Then, the daily wind speed was presented as means for the final result. Modeling method: Multiple Linear Regression Models Multiple regressions analysis was performed using Statistical Packages for Social Sciences (SPSS-21) software for Windows. The multiple regression models were formulated using an organized data - set as follows:

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4,$$

Where Y= AD/trap/day of *Glossina f. fuscipes*, X₁ = Temperature (°C), X₂ = rain fall (cm), X₃= Relative Humidity, X₄ = Wind Speed (cm/s). Generally, the coefficient of each variable represents the capacity or sensitivity of the variable. Therefore, the coefficients for two variables must show positive values in the multiple regression models. The apparent densities of the flies obtained from the survey were plotted with the one predicted by the model (predicted apparent density).

2.4 Data Management and Statistical Analysis

SPSS-20 software compatible with Windows was used for the analysis of regression statistics and for the difference between the actual apparent densities and the ones generated from the predictive models. Statistical significance was made at P≤0.05 and very significant (P≤0.01). Microsoft Excel was used for the creation of the graph.

3. RESULTS

Regression models R-square indicates the "goodness of fit" of the model given that R-square for this model is 0.891, which means that the X variables

(temperature, rainfall, relative humidity and wind speed) can explain about 89.1% of the change in Y (*G. f. fuscipes* apparent density/trap/day).

Table 1. Model summary of the predictors

Model	R	R square	Adjusted R square	Standard error (SE) of the estimate
1	0.944a	0.891	0.829	0.79361

a. Predictors: (Constant), wind speed (cm/s), rainfall (cm), relative humidity (%), temperature (°C)

Table 2. Analysis of Variance (ANOVA) test for model fitness

ANOVA(b)						
Model		Sum of squares	Df	Mean square	F	Significance
1	Regression	36.0079	4	9.02	14.321	0.02 ¹
	Residual	04.409	7	0.63		
	Total	40.488	11			

a. Predictors: (Constant), wind speed (cm/s), rainfall (cm), relative humidity (%), temperature (°C); b. Dependent Variable: AD

Table 3. Summary of results from the regression analysis

Coefficients (a)						
Model		Unstandardized coefficient		Standardized coefficient		Significance
		β	Std. error	Beta	T	
1	(Constant)	44.813	16.446	-	2.73	0.03
	Temperature	-1.153	0.568	-0.768	-2.03	0.08
	Rainfall	-0.219	0.179	-0.435	-1.22	0.26 ^{NS}
	Humidity	-12.759	4.496	-1.052	-2.84	0.03*
	Wind speed	0.774	0.765	0.332	1.01	0.35 ^{NS}

NS, Non-significant ($P > 0.05$); *Significant ($P \leq 0.05$); **Very Significant ($P \leq 0.01$); a. Dependent Variable: AD

(Table 1). The ANOVA shows that the regression model has a significant predictive value, ($F(4, 11) = 14.321$, $P < 0.02$) (Table 2).

Table 4 shows twelve regression models based on the general regression formula to forecast the effect of temperature, rainfall, humidity and wind speed on tsetse fly apparent density/trap/day. The estimated model predicted the synergistic effects of temperature, rainfall, relative humidity and wind speed on *G. f. fuscipes* AD/trap/day. Y1, Y2, Y3, Y4, Y5,....., Y12 represent the estimated apparent densities for the months of January, February, March, April, May,.....,December respectively.

Maximum ADs/trap/day of 7.71 flies were recorded from the model. The maximum ADs/trap/day was observed in January at the temperature, 28.30°C; rainfall (cm); RH%, 44 and wind speed, 1.76 cm/s. Similarly, minimum ADs/trap/day of 1.83 and 1.79 flies were recorded in September and October,

respectively. As such, the minimum ADs/trap/ day revealed at 26.8°C; rainfall, 8cm; RH, 78% and wind speed, 1.35 cm/s.

3.1 Model Summary

The model summary offers the multiple R and coefficient of determination (R2) for the regression model. R2 = 0.829 indicates that 82.9% of the variance in the fly's apparent density can be explained by the model. Hence, forecasting of the fly abundance during the period of study is strongly related to the selected environmental variables (Table 1).

3.2 Model Validation and Fitness

The overall combined linear effects of the environmental variables significantly predicted fluctuation of fly apparent density, $F(4,11) = 14.321$, $P < 0.02$. Therefore, the model has shown the power to predict the outcome more accurately than just using the means to model the data (Table 2).

3.3 Model Coefficients

The model coefficients give the constant or intercept term and the regression coefficients (β) for each explanatory variable (Table 3). The constant value (44.813) represents the intercept, which is the predicted fly apparent density score when all variables score = 0. The other value here is the regression coefficients (β) for the selected environmental variables. For every unit increase in temperature the model predicts a decrease of 1.153 in the fly apparent density score; increase in the rainfall and humidity, the model predicts a decrease of 0.229 and 12.729 in the fly apparent density scores, respectively. Whereas in every unit an increase in wind speed, the model predicts an increase of 0.774 in the fly apparent density score.

Table 4. Multiple linear regression general model outputs for *G. f. fuscipes* AD/trap/day as a function of environmental parameters

Month	GM	$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \dots + \beta_n x_n$	AT	RF	RH	WS	PAD	ACAD
Jan	1	$Y1 - 44.813 - 1.153X1 - 0.219X2 - 12.759X3 + 0.774X4$	28.3	1	0.44	1.76	7.55	8.43
Feb	2	$Y2 - 44.813 - 1.153X1 - 0.219X2 - 12.759X3 + 0.774X4$	29.5	2	0.45	1.82	5.9	5.6
Mar	3	$Y3 - 44.813 - 1.153X1 - 0.219X2 - 12.759X3 + 0.774X4$	29.9	6	0.48	2.95	4.89	4.77
Apr	4	$Y4 - 44.813 - 1.153X1 - 0.219X2 - 12.759X3 + 0.774X4$	29.2	9	0.69	3.63	3.44	3.16
May	5	$Y5 - 44.813 - 1.153X1 - 0.219X2 - 12.759X3 + 0.774X4$	27.9	11	0.74	2.45	2.63	3.34

Month	GM	$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \dots + \beta_n x_n$	AT	RF	RH	WS	PAD	ACAD
Jun	6	Y6-44.813-1.153X1-0.219X2-12.759X3+0.774X4	26.9	9	0.83	1.2	2.25	2.61
Jul	7	Y7-44.813-1.153X1-0.219X2-12.759X3+0.774X4	25.9	11	0.89	1.16	2.46	2.41
Aug	8	Y8-44.813-1.153X1-0.219X2-12.759X3+0.774X4	26.1	11	0.84	1.45	3.05	2.17
Sep	9	Y9-44.813-1.153X1-0.219X2-12.759X3+0.774X4	26.8	8	0.78	1.35	1.85	1.83
Oct	10	Y10-44.813-1.153X1-0.219X2-12.759X3+0.774X4	27.5	9	0.82	1.45	1.77	1.87
Nov	11	Y11-44.813-1.153X1-0.219X2-12.759X3+0.774X4	27.6	6	0.73	1.24	3.27	3.99
Dec	12	Y12-44.813-1.153X1-0.219X2-12.759X3+0.774X4	27.7	1	0.67	0.9	4.99	4.61

Paired samples T-test; df=11; P (2-tailed) = 0.69; GM, General Model; AT, Average Temperature (°C); RF, Rainfall (mm); AH, Atmospheric Humidity%; WS, Wind Speed (cm/s); PAD, Predicted Apparent density (Fly density/trap/day) and ACAD, actual apparent density (Fly density/trap/day)

Models predicted values that were more accurate and had indicated the trends of *G. f. fuscipes* abundance on monthly basis. The limits of agreement were calculated from $t \pm 1.96\sigma$, where 't' is the mean of difference between each pair of predicted and actual values, and σ is the standard deviation of the difference between these pairs [28]. The upper and lower limits of agreements of model were 5.97 and -11.65, respectively.

95% of datasets of the model were within the upper and lower limits of agreement, indicating a strong concordance between the predicted and actual average of monthly *G. f. fuscipes*. The t-test from the regression analysis indicates that only humidity variable ($t = -2.84$, $P = 0.03$) made a statistically significant contribution to the predictive power of the model. The apparent densities from the predictive models and the one from the survey did not vary statistically (Paired sample T-test; df=11; $P = 0.69$) (Table 4).

4. DISCUSSION

Evidence has shown that most insects respond to changes in meteorological conditions [10] and that the spatial distribution of vector-borne infections relies on environmental factors [29]. Ecological factors such as atmospheric temperature, rainfall and relative humidity might influence seasonal variations and can consequently influence fly total catches, male/female abundance, apparent density (AD) and the infection rate [30]. Tsetse fly apparent density depends on the activity patterns of each sex and such an activity in turn depends on environmental factors (hosts and weather) and the interrelationships between these factors, as well as the fly's endogenous circadian rhythm [26].

In this study, populations of *G. f. fuscipes* fluctuated in space and time as local climate changed. This is due to the fact that tsetse flies are very sensitive to environmental changes and ecological instability, and they are found in ecologically suitable habitats having necessary temperature, humidity and vegetation covers. Frequently, *G. f. fuscipes* thrives in the habitats, which are characterized by high humidity [7].

The estimated models, used a 12-month average of the predictor variables, in order to predict the apparent density of *G. f. fuscipes* in the study area. This study therefore attempts to explain fluctuations of the vector apparent density/ trap/day based on environmental predictors. Humidity contributed to the model ($P=0.03$) temperature, rainfall and wind speed did not contribute much in the model (temperature, $P=0.08$; rainfall= 0.26 ; wind speed, $P=0.35$). With the exception of wind speed, all other independent variables had negative correlation weights (negative standardized β coefficients) in the estimated regression models. Seemingly, the significantly positive value of standardized β coefficients of independent variables could indicate an increasing level of the dependent variable(s). Whereas, higher levels of the independent variables with negative correlation weights are expected to produce lower levels of the dependent variables.

Ostensibly, the maximum ADs/trap/day records for both the estimated model and for the survey occurred in January, and the least ADs/trap/day records for the estimated models occurred in October. However, for the data obtained through tsetse survey, the least ADs/trap/day records occurred in September. Occurrence of maximum Tsetse fly Ads/trap/day in January might be due to low levels of rainfall, RH and wind speed during this month. In contrast, during September and October there are high rainfall, high RH% and relatively low wind speed. The mean annual temperatures for tsetse fly shown in this study are ranging between (19-30°C). This means that temperatures less than 19°C and greater than 30°C might affect the fly in different ways and may lead to reduction in their abundance and consequently apparent density [31].

This study has shown that no temperatures below 17-20°C were observed all the temperatures observed and recorded were within the tsetse optimum range (19-30°C). As discussed above, maximum ADs/trap/day were observed in January at the optimum temperature, low rainfall level, low RH%, high wind speed, whereas minima ADs/trap/day in September were at optimum temperature, high rainfall level, high RH% and relatively low wind speed. The levels of these predictors might be responsible for *G. f. fuscipes* estimated AD peak in January.

However, *G. f. fuscipes* density was low in September due to high rainfall and RH% levels, despite the reasonable temperature level observed during September. Generally speaking, the models showed that increased rainfall and humidity could lead to the reduction in fly density. Nevertheless, rainfall does not have any direct effect on tsetse, but it does so indirectly by affecting the humidity, causing local flooding which may drown many pupae and maintaining different vegetation zones, based on quantity of rainfalls and longevity of the rainy season [32]. These factors explained above could have contributed to the low fly density in September. However, Kleynhans and Terblanche [33] have confirmed that

temperature and RH% variations in the field frequently affect the population dynamics of tsetse. This is in line with the findings of Khormi et al. [10] who also correlated tsetse distribution in Lake Victoria with environmental changes. Likewise, in KKC, variations in ecological attributes affect seasonal and population dynamics of *G.f. fuscipes* [30,34].

5. CONCLUSION

The Multiple Linear Regression Models predicted *G. f. fuscipes* apparent density/trap/day and demonstrated the effects of the environmental variables on the abundance of *G. f. fuscipes* in KKC.

The model showed no statistically significant difference between the model-driven apparent densities and the actual apparent densities from *G. f. fuscipes* survey. This indicates that the developed models are authentic to a certain extent to predict and generate information on the *G. f. fuscipes*' apparent densities just as the survey study did.

The MLRM models are powerful tools to predict *G. f. fuscipes*' fly apparent density/trap/day as a function of environmental predictors. Therefore, the models are robust and flexible and could find applications in the various aspects of tsetse studies and provide useful information for tsetse and trypanosomiasis control programmes in South Sudan.

Further studies are needed using the MLRM to predict the effects of climate change on *G.f. fuscipes* infestation rates, feeding behavior and tsetse-parasite interaction.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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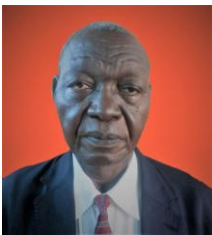
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