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Application of linear regression models in predicting the density of *Glossina fuscipes fuscipes* (Diptera: Glossinidae) in Kajo-keji County, Central Equatoria State, South Sudan

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***Glossina fuscipes fuscipes* remain the main tsetse vectors of *Trypanosoma brucei gambiense* that causes Human African Trypanosomiasis (HAT) in South Sudan, where HAT Control Strategy does not involve vector control component. Information on the fly apparent density/trap/day helps identify priority areas for vector control. Insecurity and logistic problem makes it impossible for vector control to be carried out. Fly-human contacts might be reduced in areas where the fly infestation may contribute to the disease transmission. This study employs Linear Regression Analysis to predict adult *G. f. fuscipes* apparent density/trap/day in Kajo-keji County. Tsetse field surveys were carried out along 8 streams in the study area from January 2012 to December 2012. Twelve linear regression models were developed to predict the apparent density /trap/day as function of potential predictors for tsetse fly catches. The difference between the fly apparent densities generated by the models and the actual densities from the survey was analyzed using paired samples T-test in SPSS. Models' predictive values showed the monthly trends of *G. f. fuscipes* abundance with the upper and lower limits of the model agreements of 5.97 and -11.65, respectively. 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 model did not statistically ($df=11$; $P = 0.69$) vary from the actual ones. This study could contribute to predict the peaks of the vector abundance that guide strategic plans for tsetse and HAT control programmes in South Sudan.**

Key words: *Glossina fuscipes fuscipes*, apparent density, regression models, environmental factors.

INTRODUCTION

Tsetse fly (*Glossina* sp.) is the main vector for trypanosomes, the parasites which cause trypanosomiasis and these vectors are grouped into three main subgroups, namely, the riverine subgroup known as the "palpalis", the savannah subgroup called the ''morsitans subgroup''and forest-dwelling tsetse known as the ‘’fusca’’(Wamwiri and Changasi, 2016).

Glossina fuscipes are the most important biological vectors of Human African Trypanosomiasis (HAT), in almost 90% of all disease cases across Africa (Omolo et al., 2009). Evidence has also shown that patches of *G. fuscipes fuscipes* exist on the margins of Lake Victoria in Tanzania (Krafsur et al., 2008), and in Southwestern Ethiopia and South Sudan (Rogers and Robinson, 2004). 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 (Mohammed et al., 2010; Ruiz-Postigo et al., 2012). 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 (Albert et al., 2015). River banks provide source of blood supply to tsetse from the dwellers during water collection.

Understanding how environmental factors and their drivers provide impetus for shaping tsetse (*G. f. fuscipes*) population may be crucial for tsetse control and intervention programmes. Population dynamics of the tsetse may be influenced by environmental factors/ predictors such as temperature, rainfall, humidity and wind speed. Effects of these 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 (Albert et al., 2015).

Multiple regression models are often used in many study areas using simple assumptions (Ladu et al., 2012). This model comprises both independent and dependent variables, and is easily verified, based on three viewpoints. The first is the correctness of the values predicted by the model. The second is the multi-collinearity 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 (Selvaraju et al., 2013) and adult female *Aedes aegypti* in Saudi Arabia (Khormi et al., 2013). Similarly, the model has been applied to study the potential impacts of climate change on stable flies (Gilles et al., 2008) and to predict mosquito abundance and habitats in USA (Cleckner et al., 2011).

Tsetse flies are vectors of human and animal trypanosomosis in sub-Saharan Africa and are the targets of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) (Dicko et al., 2014).

It is evidenced that the HAT remains one of the important Tropical Neglected Diseases (TNDs) threating human health in sub-Saharan Africa (Simarro et al., 2010; Mboera et al., 2011). Studies in Uganda and Democratic Republic of the Congo showed 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 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 (Bukachi et al., 2017). As a result of Glossina-borne parasite that causes HAT, it has been found that approximately 1.6 million DALYs (Disability Adjusted Life Years) is due to HAT and considered second among all vector-borne diseases in Africa for mortality and fourth for related disability (Mwiinde et al., 2017). Human African Trypanosomiasis has seriously impacted populations with greater social, cultural, and economic vulnerabilities (Holanda-Freitas et al. 2020) and has greatly affected settlements and economic developments in most African countries, particularly those south of the Sahara Desert where it is transmitted mainly by tsetse flies (Kuye, 2020).

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 (Courtin et al., 2015). Vector control programme included in the Gambian HAT control strategy in South Sudan has been initiated by PATTEC since 2009 (Rahman et al., 2010). However, the programme has never been well-implemented mainly due to insecurity problems in some foci of HAT and/or logistics constraints. Disadvantage of case detection strategy for HAT control is that the programme hardly covers more than 75% of the population and an alternate method is to eliminate the tsetse fly which transmits the parasite causing the disease (Courtin et al., 2015). 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 (Tirados et al., 2015). 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. Solution to the insufficient resource allocation for vector control programmes might

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be done by implementing the programme in certain areas where the vector control activities might achieve greater impacts. Such areas need be given priorities and this can be done on the basis of the fly apparent densities.

So far, there has no information about the apparent densities of *G. f. fuscipes* available 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 MLRM method might 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 is still 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 (Courtin et al., 2015) and this will at least prevent 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.

METHODOLOGY

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 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.

Entomological survey, 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 24 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 (Lukaw et al., 2016).

The sample size of tsetse was determined by 95% confidence interval at a desired level of 5% (Thrusfield, 1995) 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 (Challier et al., 1977). These traps were deployed 150 m apart and 5 m distant from the streams (Mohamed-Ahmad and Wynholds, 1997). 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.

Tsetse fly apparent density/trap/day

The fly apparent density/trap/day (AD) was calculated as described by Dede et al*.* (2005) as follows:

$$AD = \frac{\text{No.of the caught flies/trap}}{\text{day}}$$

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:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4$$

Where Y= AD/trap/day of *Glossina f. fuscipes*, X_1 = Temperature (^{0}C), X_2 = rain fall (cm), X_3= Relative Humidity, X_4 = 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).

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 $P \leq 0.05$ and very significant ($P \leq 0.01$). Microsoft Excel was used for the creation of the graph.

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[a]
	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*≤0.05); **Very Significant (*P*≤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. Y_1, Y_2, Y_3, Y_4, $Y_{5,\ldots\ldots,}$ Y_{12} 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 Janaury 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.

Model summary

The model summary offers the multiple R and coefficient of determination (R^2) for the regression model. R^2 = 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).

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).

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

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

Month	GM	$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \ldots \beta_n x_n$	AT	RF	RH	WS	PAD	ACAD
Jan	1	Y_1=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	28.3	1	0.44	1.76	7.55	8.43
Feb	2	Y_2=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	29.5	2	0.45	1.82	5.9	5.6
Mar	3	Y_3=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	29.9	6	0.48	2.95	4.89	4.77
Apr	4	Y_4=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	29.2	9	0.69	3.63	3.44	3.16
May	5	Y_5=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	27.9	11	0.74	2.45	2.63	3.34
Jun	6	Y_6=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	26.9	9	0.83	1.2	2.25	2.61
Jul	7	Y_7=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	25.9	11	0.89	1.16	2.46	2.41
Aug	8	Y_8=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	26.1	11	0.84	1.45	3.05	2.17
Sep	9	Y_9=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	26.8	8	0.78	1.35	1.85	1.83
Oct	10	Y_{10}=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	27.5	9	0.82	1.45	1.77	1.87
Nov	11	Y_{11}=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	27.6	6	0.73	1.24	3.27	3.99
Dec	12	Y_{12}=44.813 -1.153X_1-0.219X_2- 12.759X_3+0.774X_4	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).

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.

Models predicted values that were more accurate had indicated the trends of *G. f. fuscipes* abundance on monthly basis. The limits of agreement were calculated from t ± 1.96σ, 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 (Khormi et al., 2014). 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 predoctive models and the one from from the survey did not statistically vary (Paired sample T-test; df=11; *P* = 0.69) (Table 4).

DISCUSSION

Evidence has shown that most insects respond to changes in meteorological conditions (Khormi et al., 2013) and that the spatial distribution of vector-borne infections relies on environmental factors (Bergquist, 2001). Ecological factors such as atmospheric temperature, rainfall and relative humidity might influence seasonal variations in the fly total catches, male/female abundance, AD and the infection rate (Lukaw et al., 2014). The fly apparent density or tsetse trap catches depend on the activity pattern 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 (Mohamed-Ahmed and Wynholds, 1997).

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 cover. Frequently, *G. f. fuscipes* thrive in the habitats, which are characterized by high humidity (Albert et al., 2015). The estimated models, preceding a 12-month average of the predictor variables, have been used to predict the apparent density of *G. f. fuscipes* in the study area. This study therefore attempts to explain prediction and 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 display much contribution in the model (temperature, *P=0.08*; rainfall=*0.26*; windspeed, *P= 0.35*). With the exception of windspeed, all other independent variables had negative correlation weights (negative standardized β coefficients) in the estimated model 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 of the estimated model and of the survey occurred in January, and the least ADs/trap/day records for the estimated models occurred in October and the survey in September that could be explained by the low levels of rainfall, RH and wind speed during January. In contrast, during September and October there are high rainfall, high RH% and relatively low wind speed. The mean annual temperature (19-30°C) for tsetse shown in this study could expose the flies to temperatures greater than the above and lower than the range reported. This might affect the fly indifferent ways leading to reduction in their abundance and hence the apparent density (DeVisser et al., 2010). This study has shown no temperatures below 17-20°C which means that all the temperatures observed are within the tsetse optimum range. Seemingly, the maximum ADs/trap/day were observed in Janaury 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 (Isaac et al., 2011). These reasons could have contributed to the low fly density in September. However, Kleynhans and Terblanche (2011) have confirmed that the 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. (2013) who also correlated the tsetse distribution in Lake Victoria with environmental variation. Likewise, in KKC variations in ecological attributes affect the seasonal and population dynamics of *G.f. fuscipes* (Lukaw et al., 2014).

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 the survey. This indicates that the developed models are authentic to a certain extent to predict and generate information on the fly's apparent densities just as the survey study did.

The MLRM models are powerful tools to predict tsetse 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.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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