Effect of soil pH (Hydrogen and hydroxyl ions concentration) on the lifecycle of the *Tunga penetrans*.

Authors : J.Ngunjiri[1] , H. Ochanda[2] , F. Oyieke [3] and P. Keiyoro[4]

[1,] P.O.Box,--------, Nyeri; [2, 3] University of Nairobi, C.B.P.S. School of Biolgical Sciences, P.o box 30197, Nairobi Kenya. [4] P. O Box ----------

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Abstract

Soil pH is a measure of hydrogen and hydroxyl ions concentration in the soil. The *T.penetrans* off host stages inhabits soil during development thus soil pH is an important abiotic factor. Laboratory analysis was carried out on the soil samples collected from the field. The standard probe and meter were used in determining the soil pH. The soil pH was shown to influence the *T.penetrans* population. The soil pH of the soil samples collected was found to belong to two classes, the neutral class and slightly alkaline class. Soil pH was shown to have significant effect on the *T.penetrans* population (P=0.00) hence the prevalence of *T.penetrans* infestations.

Introduction

Tunga penetrans is believed to have been introduced in west coast of Africa in late 19th century from where the jigger flea might have been spread to the rest of sub-Saharan region (Hesse, 1899; Henning, 1904; Hicks, 1930; Jeffreys, 1952) from South America. Trade routes and military movements are said to be the way through which the *Tunga penetrans* was spread to East African region and Malagay (Blanchard 1899, Hesse 1899,; Jefferys 1952, Hoeppli 1963). Tungiasis was prevalent in Kenya during colonial period. The jigger flea might have been introduced in the country through military camps (Hoeppli 1963). It is during this period when poverty level was high and clamping of squatters in white settlers farms was rampant. *Tunga penetrans* probably was spread to different areas in the country by human movement through migration and trade routes and thrived in areas that provided good habitats.

The transmission of *Tunga penetrans* is through embedding of female *Tunga penetrans* from inhabited host dwelling or other places close to the host dwelling. This is because a large part of the life cycle of *Tunga penetrans* occurs in the soil. Hence, soil pH which is measure of acidity and alkalinity that is concentration of hydrogen ions and

hydroxyl ions (White, 1979; Brady, 1984) can influence the life of jigger flea's .Therefore, according to Piger *et al* (2008); high acidity of the soil would negatively influence the development of the *Tunga penetrans*. He found that in the households where the soil pH had been reduced due to the concentration of pig urine the absence of *Tunga penetrans* was noted.

***T.penetrans* lifecycle.**

Tunga penetrans undergoes complete metamorphism in its life cycle, which comprises of egg, larvae, pupa and adult (Geigy and Herbig, 1955). The life cycle takes about eighteen days when the conditions are favourable, eggs are dropped on the ground and hatch into larvae. The larva stage has two instars, which can be prolonged by unfavourable conditions such as low temperatures and lack of food. Pupa stage begins when larva spins into a cocoon using silk produced from its salivary glands. The cocoon is camouflaged by dust particles and organic debris, thus it is not easily picked from the habitat.

Adult *T.penetrans* emerges from cocoon when stimulated by various stimuli in the habitat for example the movements of the host, carbon dioxide from the host and probably changes in relative humidity. The Adults may survive for several months in cocoons especially in cases where the houses have been vacated for a long time. The adult hence emerge from the cocoon only when the dwelling places are reoccupied. This is one of the adaptations of the flea for survival. This also explains why when houses are reoccupied the occupants immediately are infested by the bloodthirsty fleas. *T.penetrans* feed on blood of humans probably being the main host.

The mating is said to take place when the female burrows in the epidermis and gametes stored in spermatheca (Geigy, 1953; Geigy and Suter, 1960) however mating has also been said to occur before penetration into the host epidermis (Bruce et al, 1942; White, 1987). The embedded female *T.penetrans* continues feeding on blood meal. The abdomen enlarge due to developing eggs hence the *T.penetrans* becomes gravid. The gravid female lays the eggs through the opening on surface of the host's epidermis and the lifecycle goes on as shown in figure 2.

Materials and methods

Study area

Study area was Gaichanjiru location in Kandara division of Murang'a county, Kenya. This area can be considered a "hot spot "of the *Tunga penetrans* infestations. According to 1999 census the larger Murang'a had a population of 348,304 people. The area falls under two main agro ecological zones upper midland zone (UM2) which is a main coffee zone and upper midland zone 3(UM3)which is a marginal coffee zone. Altitude of the area is between 1340m-1670m, annual mean temperatures range from

20.7 to18.8 degrees Celsius and annual average rainfall is 900mm-1620mm. The major part of this county has soils with variable topsoil which is fairly rich in organic matter (Farm management hand book of Kenya, 1979).

Gaichanjiru location is characterized by basaltic agglomerates rocks(Fairburn ,1965) which falls on Fort Hall area latitudes 0° 30' and 1° 00's longitudes 37° 00' E and 37° 30' E. These are igneous rocks which contain porpohyritic felspar basalts and melanocratic basalts. In addition, Gaichanjiru is characterized by unreliable source of water and

mainly rely on streams, which are seasonal.

Study design.

This was a cross section study. This included collecting samples for analysis in the laboratory and macroscopic examination of *T.penetrans* lesions in human beings that were manifested by embedded *T.penetrans* at different stages. The lesions on human beings were examined on hands, legs and other parts that are not humiliating to the person being examined. Sixty households were identified using random selection from the five sub locations.

Prevalence was established per household. All individuals in randomly selected homesteads were examined for tungiasis through macroscopic examination, which was confirmed by embedded *T.penetrans* which were at different stages of development. The severity of infestation was categorized into three severe (>30 lesions), mild (10-30 lesions), low (<10 lesions).

Ethical considerations.

The research required ethical measures that protected the individuals who were part of the research. This was of paramount importance to the researcher. This is because, the residents were very sensitive to people who were looking at tungiasis in the area .In fear of the information obtained would be used to get money, which would benefit the researcher.

During the preliminary study, the objectives of the study were explained to the residents of the study area. Research permit was obtained from department of development and research in the Ministry of Higher Education. Before the study commenced the permit was presented to local authorities who provided further authorization to undertake research in their administrative areas. Privacy and confidentially was taken into account.

The collection of the soil samples from the field.

Soil samples were collected by simple random selection of the households in the area of study. This was done through use of random numbers generated. Samples were

collected from inside the houses. Number of households per sub-location as shown in Table 1.

Results

Table 1: Number of households per sub location.

Sub location	Population	Proportion	Number of households
Kagira	11,780	0.150	9
Ngurwe-ini	20,167	0.256	15
Gaichanjiru	14,580	0.185	12
Maria-ini	18,834	0.239	14
Kagumo-ini	13,430	0.170	10
Total	78,791	1	60

The soil samples were collected between December 2009 and April 2010.
Each sample was transferred in poly pot that had a capacity of 30cm cubed from an area of 10cm squared.
Soil samples were analyzed individually. The larvae were isolated under a dissecting microscope. This was done immediately after the samples were transported from the field.

Determining the prevalence of *Tunga penetrans* infestations and population.
The rate of infestation was established by counting the number of lesions of *T. penetrans* infestations per individual in the selected households. The intensity of infestation was expressed; zero to ten lesions was categorized as low infestation, ten to 30 lesions moderate and above thirty lesions severe.
Prevalence per household was calculated as the number of individual infested divided by the total number of people present multiplied by 100.

Number of larvae in a given soil sample was used as indicator of the *T.penetrans* population. It should be noted that the larva stage was preferred to indicate the *T.penetrans* population since adults are highly mobile hence cumbersome to trap and are only one percent of total population (Linardi and Guimarães, 2000). The pupa stage

is quiescent and camouflaged in the habitat. The pupa stage is also known resistant to harsh conditions as well as insecticides. The eggs viability to hatch could not be determined hence this delimited use of the eggs as an indicator of the population.

Isolating *T.penetrans* larvae from the soil was undertaken as follows.

The soil samples were transferred into Petri dish in small amounts from the poly pots. The Petri dishes were placed under the dissecting microscope and a wooden splint was used to stir the soil under magnification of X15.The larvae were then isolated from the soil into a clean poly pot using the spatula. The larvae moved actively from the light. Some of the larvae were preserved in formalin and mounted on a microscope slide using Hoyer's medium. The rest were placed in poly pots containing small amount of soil for monitoring them pupate. Unfortunately, they died within a short time and none pupated. The number of the larvae isolated from each sample was recorded to determine the population.

Determining the soil pH of the soil samples.

PH is a measure of the hydrogen ion concentration [H+] (Brady, 1984).In acid classes' concentration of hydrogen ions is higher than in basic classes that have more of hydroxyl ions (Donald, 1976; Brady, 1984; White, 1979). They have classified soil pH into different classes. For example nine classes as follows; extreme acid 3.5 – 4.4, very strong acid 4.5 – 5.0, strong acid 5.1 – 5.5, moderate acidic 5.6 – 6.0, slight acidic 6.1 – 6.5,neutral 6.6 – 7.3, slightly alkaline 7.4 – 7.8, moderately alkaline 7.9 – 8.4, strongly alkaline 8.5 – 9.0(Brady, 1984).

The pH meter with probe was used (pH meter 3071, JENWAY made in the UK). The pH meter was buffered using buffer solution. The soil pH was measured using a standard probe which, was immersed in the filtrate and reading done on the meter.

Procedure for determining the soil pH.

Ten grams of each soil samples were placed in a beaker containing 10cm cubed of distilled water in a beaker. The mixture was vigorously shaken for five minutes. The mixture was then filtered using a filter paper number one with pores measuring 30microns.The probe was inserted in filtrate and reading done on the pH meter. The soil pH of the soil sample was then recorded.

Influence of soil pH on the *Tunga penetrans* population.

The soil pH was found to have an effect on *T.penetrans* population. Figure 2 shows soil pH in the households sampled.

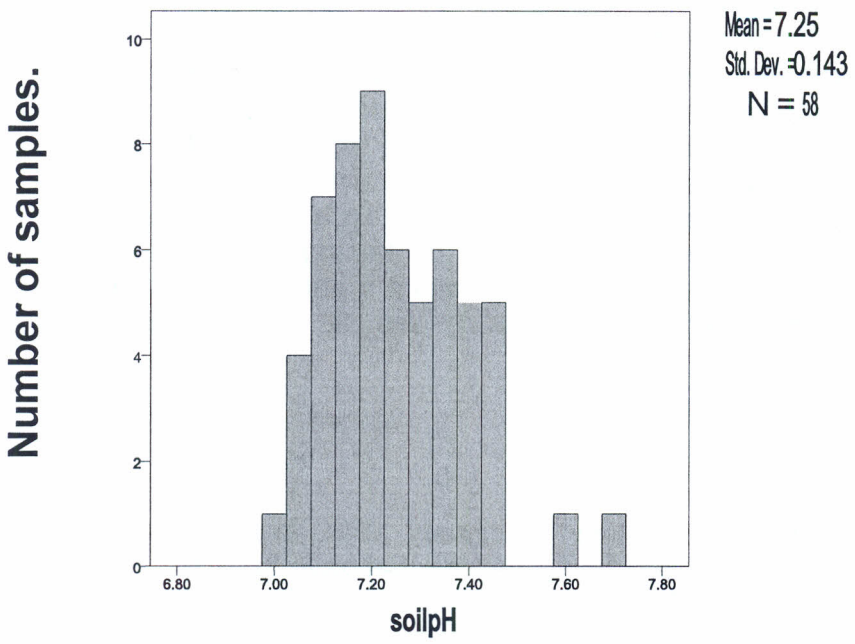


Figure 2: soil pH of the soil samples obtained from the households.

The effect of the soil pH on *T.penetrans* population was significant (P=0.000) which is less than P= 0.05. Thus null hypothesis was rejected which was that soil pH does not influence *T.penetrans* population. The alternative hypothesis was accepted that soil pH influences *T.penetrans* population. The soil pH however did not indicate significant influence on the prevalence of tungiasis (P=0.532) value which was slightly greater than P= 0.05. This was emphasised by the variability of soil pH and *T. penetrans* population which was 25.4 % (r^2=0.254) as shown in figure 3 compared to negligible value of 0.0053% (r^2 =0.000053) between soil and tungiasis prevalence as shown in figure 4.

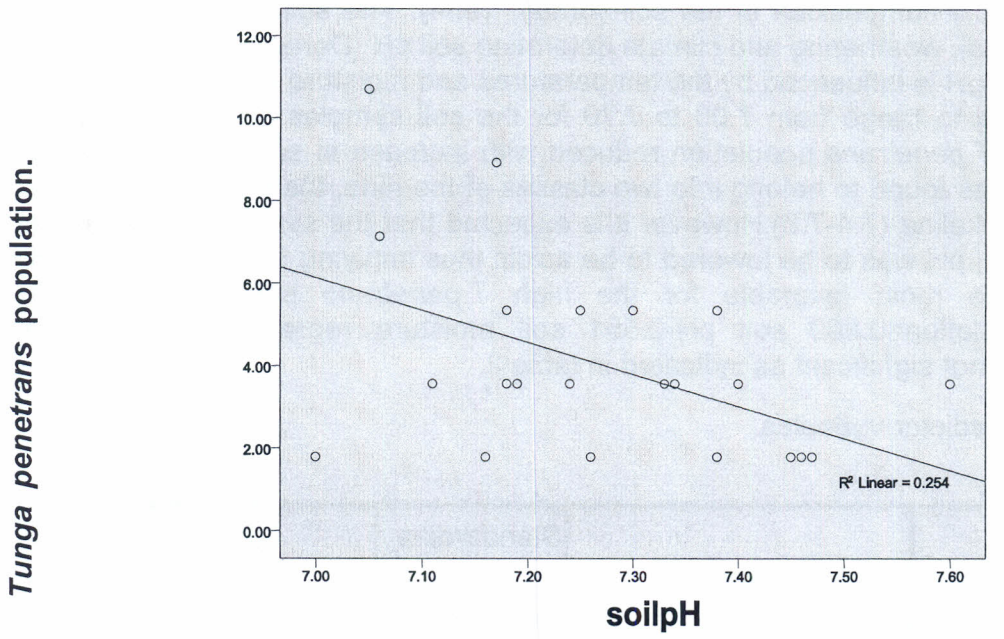


Figure 3: Relationship between soil pH and *Tunga penetrans* population.

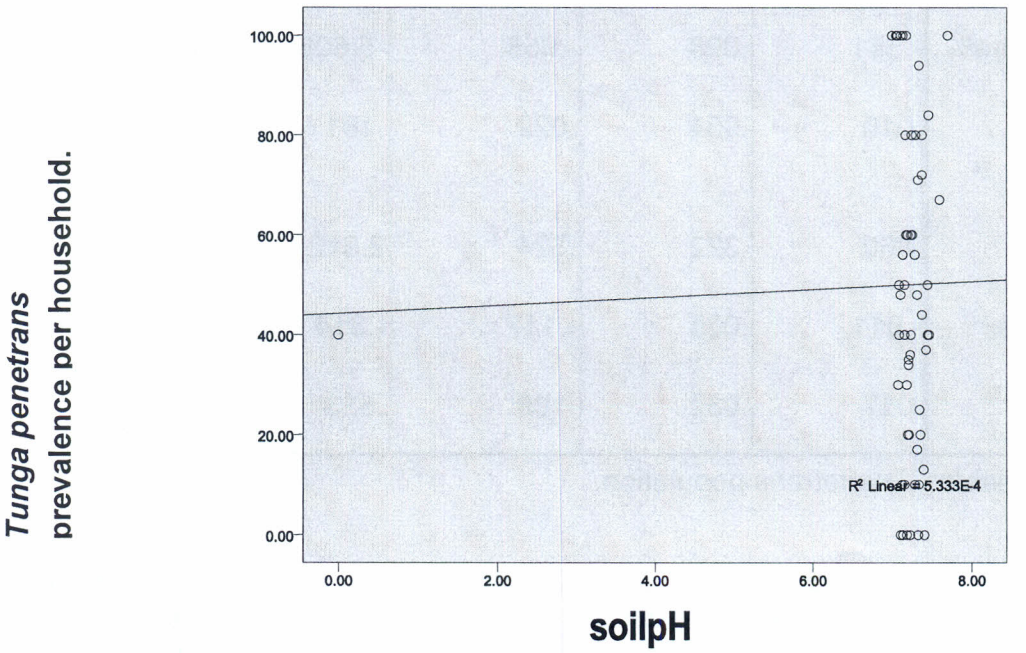


Figure 4: Soil pH relationship with the prevalence of tungiasis.

The relationship between the soil pH, the *Tunga penetrans* prevalence and population.

The pH influences microbial activities, which in turn affect decomposition of organic matter and chemical composition in the soil (Brady, 1984). The soil minerals resulting from parent material, weathering and climate determine soil pH (Donald, 1976). He also noted that the soil pH is influenced by the temperatures and moisture content.

Soil pH was found to range from 7.00 to 7.70 for the soil samples obtained from the households. The *T.penetrans* population reduced with increase in soil pH from neutral pH. The soil pH was found to belong into two classes of the nine, the neutral class (6.6-7.3) and slightly alkaline (7.4-7.8).However it is expected that the same trend would be observed if the soil pH was to be lowered to be acidic thus implying that the neutral soil pH would be the most favorable for the high *T.penetrans* population density. *T.penetrans* population=0.860 soil pH-0.551 soil moisture regression model, the constant (B_o) was not significant as indicated in table 2.

Table 2: Model predictor variables.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.165	2.186		.075	.940
	Soil moisture%	-.551	.098	-.658	-5.608	.000
	Organic content%	.010	.054	.022	.181	.857
	Soil pH	.860	.322	.324	2.672	.010
	Soil texture	-.011	.035	-.117	-.314	.754
	Soil colour	.017	.032	.199	.533	.596

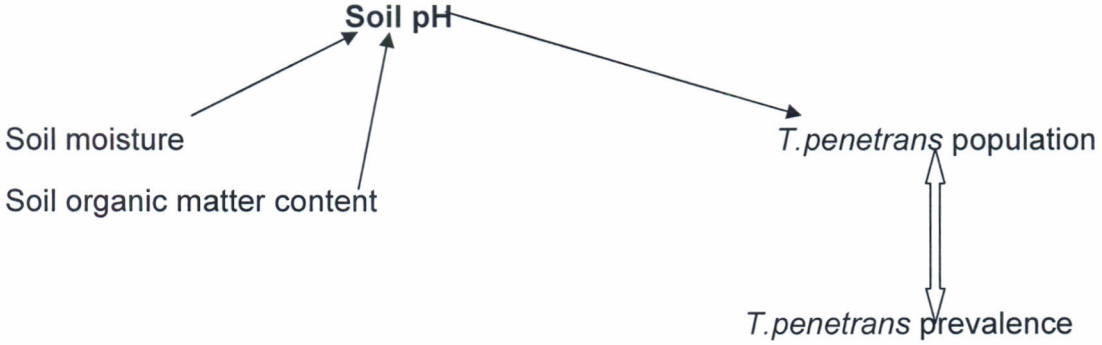
a. Dependent Variable: *T.penetrans* population.

Regression model , *T.penetrans* population=0.872 soil pH - 0.560 soil moisture showed that the soil pH was crucial independent predictor of the *T.penetrans* population.

The soil pH increased from neutral to slightly alkaline probably due to wood ashes from the cooking area. Soil pH fluctuations beyond the optimum range would cause osmotic

imbalance that would therefore lead to death. Water is buffer that is it will release hydroxyl ions to combine with hydrogen ions if the pH lowers and vice versa. However it can only buffer up to certain degree of the hydrogen and hydroxyl ions fluctuations. Thus water in an organism cannot fully control osmotic imbalance caused by the pH changes especially if the deviation is more than the optimum range. The soil pH would influence the pH of the *T.penetrans* off-stage inhabiting that particular soil. Piger *et al.,* (2008) found that high acidity of the soil caused negative effect on the development of *T.penetrans*.

The soil organic matter acts as buffer for the soil pH (Donald, 1976; Brady, 1984; White, 1979). Thus, the organic matter content may be affecting *T.penetrans* population indirectly. The soil organic matter in the soil would influence the amount and duration of soil moisture present at any given time. Thus in endemic areas organic matter should not be ignored however, the nature and type would be of greater importance especially in the households and reservoirs burrows.



Thus soil pH deviations from optimum range would cause death to larvae and probably corrode the eggs lowering the number that would hatch into larvae. This would lead to reduced *T.penetrans* population which would mean that prevalence would also be low.

Discussion:-

Conclusion and Reccommendations

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REFERENCES

Blanchard ARAE (1899) Présence de la chique (*Sarcopsylla penetrans*) à Madagascar. *Archives of Parasitology* 2 , 627–630.

Brady Nyle E.(1984) The nature and properties of soils 9th edition Macmillan publishing company.

Donald Steila(1976);The Geography of soils. Prentice –Hall, Inc. Englewood cliffs, New Jersey.

Hesse, P. (1899) Die Ausbreitung des sand fleas in Africa . Geographische Zeitsrift (Hettner), 522-530 Volume II Part B (1979) ;Farm management hand book of Kenya. Central Kenya.

Henning G (1904) Zur Geschichte des Sandflohs (*Sarcopsylla penetrans* L.) in Afrika. *Naturwissenschaftliche Wochenschrift* 20 , 310–312. 35 Hicks EP (1930) The early stages of the jigger, *Tunga penetrans*. *Annals of Tropical Medicine and Parasitology* 24 , 575–586

Hoepplli R. (1963)Early references to the Occurrence of *Tunga Penetrans* in Tropical Africa. Acta Tropical 20 : 143-53

Fairburn W.A. (1965) Geology of the Fort Hall, Degree Sheet 44,S.W. Quarter pg13-16

Jeffreys MDW (1952) *Pulex penetrans*: the jigger's arrival and spread in Africa. *South African Journal of Science* 48 , 249–255.

Linardi, P. M. and L. R. Guimaraes(2000) Sifonápteros do Brasil. São Paulo: *Ed. MZUSP, FAPESP*, São Paulo, Brazil. 291 pp.

Piger Daniel,Schwalfenberg Stefan,Heukelbach Jorg,Witt Lars,Mehlhorn Heinz,Mencke Norbert, Khakban Adak, Feldmeier Hermann(2008) Investigations on the biology, epidemiology, pathology and control of *Tunga penetrans* in Brazil;VII The importance of animal reservoirs for human infestation.*Parasitol Res* 102:875-880

White R.E. (1979) Introduction to the principles and practice of soil science .Blackwell Scientific Publications Oxford London Edinburgh.