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# Relationship between Size of Fish, Temperature and Parasitic Intensity in Snakehead Fish Species from Kepala Batas, Penang, Peninsular Malaysia

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## ABSTRACT

Acanthocephala parasite infestation was found to be high in wild Snakehead fish (*Channa striata*) from a Kepala Batas paddy field in Penang, Peninsular Malaysia. The fish parasites collected from 98 out of 100 fish samples were examined. The study showed infestation prevalence rate of 98%. Further analysis was carried out to correlate the parasitic intensity, fish size, and temperature, while statistical model summary was produced using SPSS version 15 statistical software. Statistical model summary concluded that for every increment of 1 cm in length of fish, an extra of 0.5 ≈ 1 number of parasites was found in the fish. This is only true if the effect of mean temperature remained constant. Meanwhile, an increase of 1°C is associated with a decrease of 1.487 ≈ 2 numbers of parasites in fish. This is only true when the effect of fish length remained constant. Therefore, overall model summary is described as: *Number of Acanthocephala parasites = 34.74 + (0.462*Length of Fish) + (-1.487*Mean Temperature)*.

*Keywords:* Acanthocephala parasite, Predictor, Snakehead fish, Variance Inflation Factor (VIF)

## INTRODUCTION

Malaysia is a country which depends on fisheries as one of its economic resources. Fishery not only serves as one of the most important protein supplies in Malaysia but it also helps in increasing its national Gross Domestic Product (GDP) and foreign exchange. Apart from that, the Fishery sector in Malaysia serves as an economic source of job opportunities for many people in this country, especially in rural area either as fisherman or fish farmers (Kabata, 1986; Othman, 1998).

Snake head fish (*Channa* sp.), which is more commonly known as Haruan, has

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been acknowledged in Asian countries as one of the most important fish species. Snake head fish (*Channa* sp.) is commonly found in the wild rice fields, ponds and wells distributed throughout Asia. Moreover, the feeding habits of wild snake head fish are carnivorous as the animal feeds on worms, prawns, frogs and especially other fishes (Mat Jais *et al.*, 1994).

The main reason for the popularity of this species is its medical values with antinociceptive properties. It has been considered as a very good source of healthy food among Asians because of the high levels of amino acids and fatty acids (Mat Jais *et al*., 1994). Although there are numerous advantages of snake head fish, there are also several parasites identified to be present in this fish species. The parasites present may or may not cause illness, and this characteristic is governed by various factors. Parasites have arisen by evolution from free living animals, whereby some of them have developed special organs to be able to live in host organisms (Yalcin *et al*., 2002).

The common disease recorded in snake head fish species throughout Asia is mostly on the wild type of fish from endoparasites of Acanthocephalans that are commonly found in the intestinal of fish. The lifecycle of Acanthocephalans involves invertebrates as an intermediate host and vertebrate as the final host. The phylum of Acanthocephalans consists of 4 classes: Archiacanthocephala, Palaeacanthocephala, Eoacanthocephala and Polyacanthocephala. This classification is based mainly on their morphological features such as the location of the lacunar system (network of cavities in the epidermis), the persistence of ligament sacs in females, the number and shape of cement glands in males, the number and size of proboscis hooks, as well as host taxonomy and ecology (Crompton, 1985).

To date, there has been no reference available on the correlations of Acanthocephalans infestations to the fish size and environmental factors such as water temperature in Malaysia. There is an urgent need for this important aspect of research as it will benefit fish farmers for aquaculture industry to predict any Acanthocephalans fish parasite infestation in their farm and to take initiatives to prevent intestinal parasite infections. Thus, the objective of this study was to determine the prevalence and statistical analysis of Acanthocephalans parasite to the fish size and water temperature of wild snakehead fish sampled from paddy field in Kepala Batas, Penang, Peninsular Malaysia.

## MATERIALS AND METHODS

The experiment was carried out with 100 specimens of wild *Channa striatus* from Kepala Batas, Penang (latitude 5.51531, longitude 100.41627), Peninsular Malaysia. The length (cm) of each fish was measured prior to parasite examination. Tricaine methane sulfonate (MS 222) (50 mg/L) was used as anesthetics to reduce the stress, and for easy handling. After the fish had been anaesthetized, presence of endoparasite was examined via disection of fish intestine and direct observation under light microscope.

The fish abdominal wall was pierced using a sharp pointed scissor in the mid ventral line, just behind and between pectoral fins and cut along the mid ventral line toward the anal region. Then, the belly flap was removed and this exposes the internal organs. All the internal organs like the heart, liver and gut were carefully taken out as a unit by making a transverse cut from the mouth to the anal region. Then, these organs were separated and each was placed in a different petri dish. These organs were then added with a few drops of saline.

Then, the heart, liver, spleen and kidney were dissected and observed for any abnormalities. Any white spot found was carefully cut to check on the possibilities of the parasite infestation. These organs were cut into thin slice and observed under microscope using 10x and 40x magnification. Then, the heart, liver and kidney squash were prepared and observed under a microscope. Next, the gastrointestinal tract was removed and cut from the posterior end to open it up. The intestine is dissected under dissecting microscope. The intestinal wall is scraped and their content is diluted with saline in a bottle. These diluted intestinal contents were shaken and was checked for the presence of any parasite that had settled at the bottom of the bottle. Then, the intestinal walls were carefully examined under a microscope for the attachment of the parasites (Kabata, 1986).

First, the morphological identification of parasite was done by first staining the parasite with a few drops of lactophenol solutions (200 mL lactic acid, 200 g/L phenol, 400 mL glycerol and 200 mL deionized water). Upon staining, slides were observed under the compound microscope (Leica USA). Second, the morhological identification was done using the Supra 50vp ultra high resolution LEO analytical Fesem, Scanning electron microscope. Parasite found was taken out carefully from the infected area, and the number of parasites obtained from each fish was recorded and preserved with 70% ethanol solution in universal bottle for further examination (Lasee, 2004). After taking pictures of the parasites, identification of the parasites collected was done by morphological observation using identification keys, as suggested by Hoffman (1970).

Electron microscopic sample preparation was done as suggested by the protocol of Supra 50vp ultra high resolution LEO analytical Fesem, Scanning electron microscope guide manual. Firstly, the suspended samples in ethanol were put into serial dilution of 90%, 80% and 70% ethanol. Then, a droplet of the suspension was placed on a carbon film coated with 400mesh copper grid for 1-3 minutes. The droplet was then wicked to dryness using pieces of filter paper. The grid was then placed in a filter paper lined petri dish for preservation in desiccator. Finally, imaging would be carried out after 3 days of preservation.

The statistical analysis in this study was performed using the Statistical Package for Social Sciences software 15.0, SPSS version 15, using the analysis of multiple regressions. In all cases, the level of significance was set at $p< 0.05$ (Field, 2005). Weather report of Kepala Batas Region in Penang, Peninsular Malaysia was collected from Malaysian Meteorology Department, while the statistical analysis was performed with SPSS version 15, using analysis of multiple regressions. In all cases, the level of signicifance was set at $p< 0.05$ (Field, 2005).

Note:
* Prevalence is defined by:

$$\frac{\text{Number of fish infected}}{\text{Total number of fish}} \times 100\ \%$$

(Poulin *et al*., 1995)

**RESULTS AND DISCUSSION**

Acanthocephalan parasite was observed with few body segments. Proboscis is a hollow structure filled with fluid. These fluid associates with the proboscis inverter muscles help in invagination of the proboscis into the proboscis receptacles. The movement of proboscis is activated by the apical sensory organ located at the tip of the proboscis (see Fig.1-1.3). These proboscises have a variety of shapes ranging from spherical to cylindrical (Bush *et al*., 2001). Proboscis has a proboscis sheath or septum that separates its cavity from the pseudocoelom. Their proboscis is armed with a set of chitinised pointing hooks that are arranged in horizontal rows piercing the intestinal tissue of the infected animals, resulting in inflammation at the attachment site. These hooks are usually longer and slender at the length of the proboscis but shorter at the base (see Fig.1-1.3). Some of the Acanthocephalans can enter abdominal cavity by inserting their proboscis in the host intestine. The action of this highly pathogenic parasites cause inflammation at the attachment site and severe infection that can lead to death (Kabata, 1985; Crompton & Nickol, 1985; Hoffman, 1970).

Typically, wild animals are exposed to a diversity of parasitic species including nematodes, cestodes, trematodes and acanthocephalans, representing a various group of transmission strategies and effects on host's health (Poulin, 1998b; Morand, 2000; Roberts *et al.*, 2002). An extensive approach is needed to understanding the parasite community diversity because the multiple host characteristics like environment temperature, host length may be correlated with one another, and different host characteristics may be important for understanding the parasite species correlations (Nunn *et al.*, 2003).

In this study, the multiple regression analysis was used to examine the multiple factors that had been predicted to influence the diversity of parasites in wild hosts like their length size, environment temperature and parasites count (Nunn *et al.*, 2003). These variables are predicted to influence host encounter rates with parasites in the wild, and the number of parasite species that can persist in populations. A positive association is expected between host length and parasite diversity because larger hosts represent larger habitats that provide more niches for colonization (Kuris *et al.*, 1980; Poulin, 1995; Gregory *et al.*, 1996).

The multiple regression analysis is incorporated with the aim to produce a model that would best predict on the optimal number of Acanthocephalan parasitic infestation based on the observed values of two independent variables which was the length of the fish and the mean temperature. Along with that, the multiple regression analysis also produces unique contribution on the length of fish and mean temperature variables on the number of Acanthocephalan parasitic infestation in fishes. This determines the effects of the fish's length and the mean temperature on the number of Acanthocephalan parasitic infestation.

A total of 98 out of 100 fish were found to have been infected by Acanthocephala parasite with the prevalence of 98%. Table 1 shows the descriptive statistics of

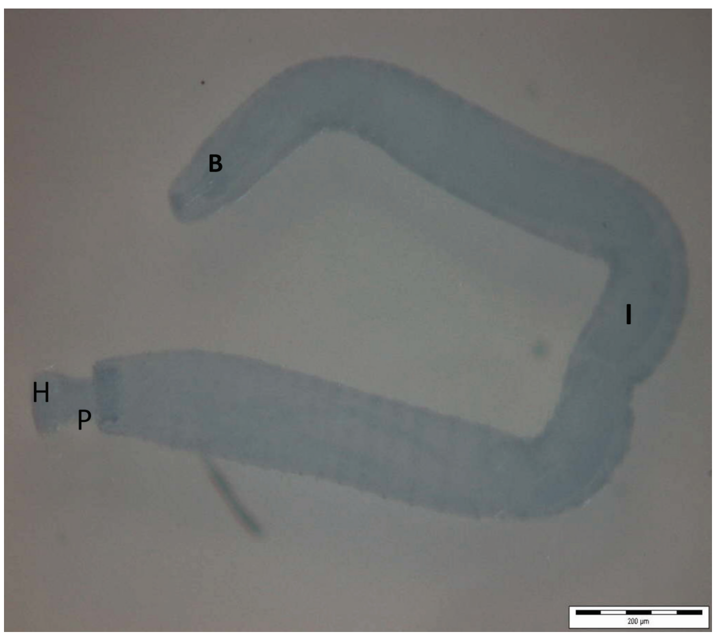


Fig.1: The morphology of Acanthocephala parasite sample collected; B= bursa, I-internal organs, H-hooks,P-proboscis

TABLE 1
Descriptive statistics of variables

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Acanthocephala Parasite | 98 | 0 | 14 | 4.82 | 2.902 |
| Length Fish | 98 | 18.00 | 38.00 | 23.0969 | 4.14844 |
| Mean Temperature | 98 | 25.60 | 28.80 | 27.3721 | .71921 |
| Valid N (listwise) | 98 | | | | |

TABLE 2
Correlation between the variables

| | | AcanthocephalaParasite | Length Fish | Mean Temperature |
|---|---|---|---|---|
| Pearson Correlation | Acanthocephala Parasite | 1.000 | .850 | -.774 |
| | Length Fish | .850 | 1.000 | -.667 |
| | Mean Temperature | -.774 | -.667 | 1.000 |
| Sig. (1-tailed) | Acanthocephala Parasite | . | .000 | .000 |
| | Length Fish | .000 | . | .000 |
| | Mean Temperature | .000 | .000 | . |
| N | Acanthocephala Parasite | 98 | 98 | 98 |
| | Length Fish | 98 | 98 | 98 |
| | Mean Temperature | 98 | 98 | 98 |

TABLE 3
Regression model summary

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate | Durbin-Watson |
|---|---|---|---|---|---|
| 1 | .894(a) | .800 | .793 | 1.303 | 2.024 |

a Predictors: (Constant), Mean Temperature, Length Fish
b Dependent Variable: Acanthocephala Parasite

the dependent variable and explanatory variables involved in this study. The average number of the Acanthocephala parasites found in dissected fishes was approximately 5, with a standard deviation of 2.902. The mean values of the parasite, fish length, and temperature were 4.23, 23.10 and 27.37, respectively.

Table 2 shows the correlation between the pairs of variables. It is evident that high positive correlations exist between the number of Acanthocephala parasite and the length of fish, which is 0.850 at 0.05 significance level, whereby p-value is 0.000. Among all the predictor variables, the length of fish correlates best with the dependent variable and therefore, it is likely that this variable will best predict the number of Acanthocephala parasite in fishes. High negative correlation between the mean temperature and number of Acanthocephala parasite, with a correlation value of -0.774, is significant at 0.05 significance level. Thus, it can be concluded that there is no multicollinearity in this data due to no substantial correlations between the predictors' value higher than 0.9 (Field, 2005).

The Table 3 shows the correlation value between the length of fish and mean temperature with the number of Acanthocephala parasites found in fish, which is 0.894. The value of R Square is 0.800, indicating that the length of fish

TABLE 4
ANOVA

| Model | | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 440.206 | 2 | 220.103 | 129.680 | .000(a) |
| | Residual | 110.323 | 65 | 1.697 | | |
| | Total | 550.529 | 67 | | | |

a Predictors: (Constant), Mean Temperature, Length Fish
b Dependent Variable: Acanthocephala Parasite

TABLE 5
Coefficients for model

| | | Model | | |
|---|---|---|---|---|
| | | 1 | | |
| | | (Constant) | Length Fish | Mean Temp |
| Unstandardized Coefficients | B | 34.741 | .462 | -1.487 |
| | Std. Error | 9.018 | .057 | .297 |
| Standardized Coefficients | Beta | | .601 | -.373 |
| T | | 3.852 | 8.063 | -5.006 |
| Sig. | | .000 | .000 | .000 |
| 95% Confidence Interval for B | Lower Bound | 16.730 | .348 | -2.080 |
| | Upper Bound | 52.751 | .576 | -.894 |
| Correlations | Zero-order | | .850 | -.774 |
| | Partial | | .707 | -.528 |
| | Part | | .448 | -.278 |
| Collinearity Statistics | Tolerance | | .555 | .555 |
| | VIF | | 1.002 | 1.002 |

a Dependent Variable: Acanthocephala Parasite

and mean temperature account for 80.0% of the variation in the number of parasites. The difference between the adjusted R Square and R Square is small, i.e. about 0.007 or 0.7%; this shrinkage defines that if the model was to be derived from the whole population rather than a sample, it would account for approximately 0.7% less variance in outcome. The Durbin-Watson value is 2.024, which is not greater than the value 3 nor is it lesser than the value 1. This only proves that the assumptions of independent errors are tenable and the assumptions have been met (Field, 2005).

Table 4 shows F-ratio is 129.680, which is very unlikely to happened by chance due to a significant p-value lesser than 0.05 or 5% significance level. This result proves that the model significantly improves the ability to predict the outcome variable. Therefore the regression model overall predicts the number of the Acanthocephala parasites in fish significantly well. Table 5 presents the estimates of the b-values, which are the parameters in the regression model, and the individual contribution of each predictor to the model. The b-values represent the parameter estimates of the model for each independent variable. For this data, the length of the fish has a positive b-value indicating that there is a positive relationship between the numbers of the Acanthocephala parasite and the length of fish. As the length increases, the number of Acanthocephala parasite also increases. However, the mean temperature has a negative b-value representing a negative relationship between the numbers of Acanthocephala parasite and the mean temperature (Field, 2005).

The overall model can be defined as follows:

Y=b0+b1x1+b2x2
Number of Acanthocephala parasites = 34.74 + (0.462*Length of Fish) + (-1.487*Mean Temperature)

The b-value for each predictor affects the outcome variable, if the effects of all other predictors are held constant (Andy, 2005). This is further explained as the length of Fish (b = 0.462) value indicates that as the length of fish increases by one unit, the number of parasites will increase by 0.462 unit. In other words, for every increase of 1 cm in the length of the fish, an extra of 0.5 ≈ 1 number of parasites is found in it. This is only true if the effect of the mean temperature remains constant. Next, the mean temperature (b = -1.487) value indicates that as the mean temperature increases by one unit, and the number of parasites decreases by -1.487 units. Therefore, an increase of 1°C is associated with a decrease of 1.487 ≈ 2 numbers of parasites in the fish. This is only true when the effect of the length of the fish remains constant.

For this model, the t-test associated with a b-value which is significant shows that the explanatory variables are making a significant contribution to the model. The length of fish has the value t-statistics of t (65) = 8.105 at a p- value < 0.05 and the mean temperature has the value of t (65) = -5.006 at a p-value < 0.05, and they are all significant predictors of the number of parasites in the fish. The magnitude of the t-statistics shows that the length of the fish has a higher impact on the number of parasites compared to the mean temperature. The model does not have confidence intervals that cross the value zero, proving that the model is quite a good model. The 95% confidence intervals for the independent variable mean temperature is small, indicating that the estimates for the current model are likely to be the representative of the true population values.

The confidence interval for the length of the fish is wider, however, did not cross zero, indicating that the parameter estimate for this variable is less representative, but it is significant. From Table 5, it can be concluded that the assumption of no multicollinearity is true due to the values of VIF and the tolerance statistics, whereby the value of largest VIF value does not exceed 10 and tolerance statistics are above 0.2.

Fig.2 shows the histogram of the residuals data. The histogram has bell shaped curve which represents that all residuals are normally distributed. Fig.3 shows the normal P-P plot and it shows that the points lie along a diagonal line indicating residuals are normally distributed. Fig.4 shows that the points are randomly and evenly dispersed throughout the plot. This pattern shows that the assumptions of linearity and homoscedasticity of the residuals have been met (Field, 2005).

## CONCLUSION

In conclusion, length of the fish and temperature are the main factors that influence the number of fish parasites present in snake head fish (*Channa* sp.). Eventually, this study has focused on predictions of several parameters so as to discover the closest correlations among the abiotic and biotic factors. The effets of temperature in this study shows a decreasing value when the number of parasites increases accordingly with the length of fish, immunological studies have suggested an immune suppression effect associated with a decrease in water temperature (Bly *et al*., 1992). Several studies have reported that a decrease in water temperature may cause suppression of acquired immunity, with components of innate immunity being relatively independent of water temperature (Magnadottir *et al*., 1992). Furthermore, there are some suggested studies which show that larger fish tend to harbour more parasites as compared to smaller ones (Rahman & Saidin, 2012).

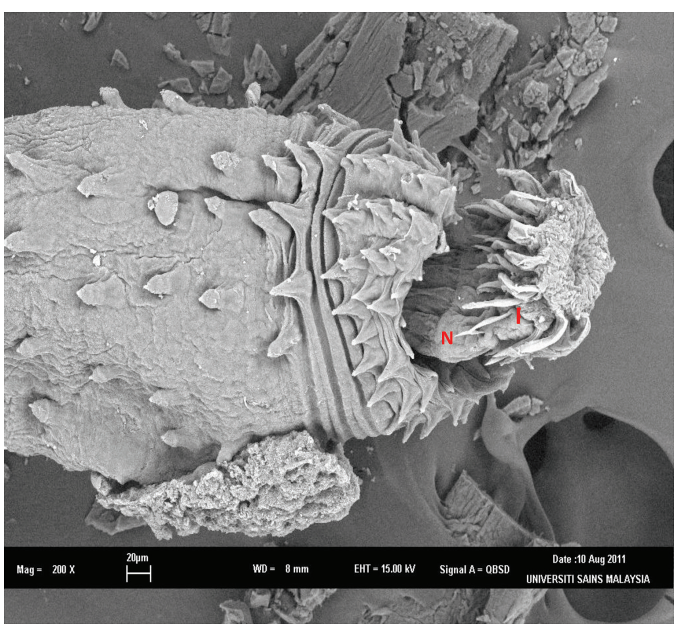


Fig.2: The upper portion view of the Acanthocephala parasite sample collected; P= proboscis, N= neck.

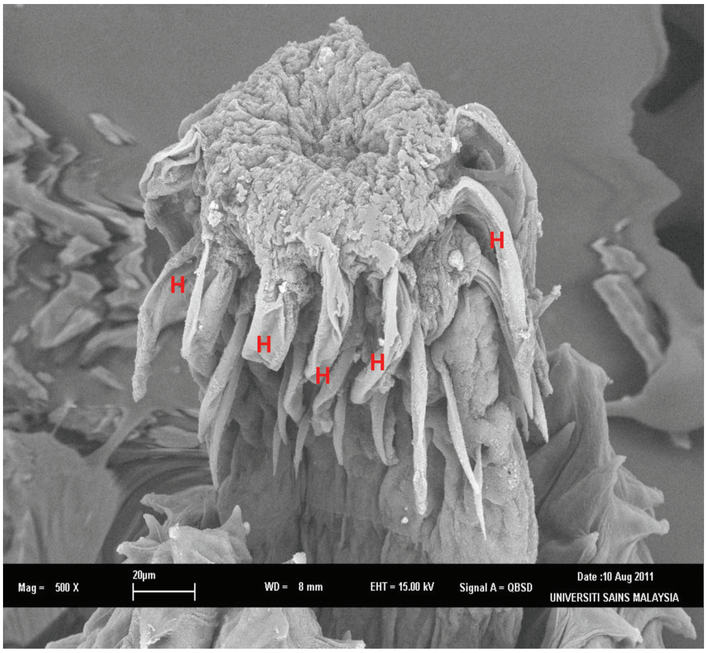


Fig.3: Anterior view of Acanthocephala parasite sample collected; H=hooks

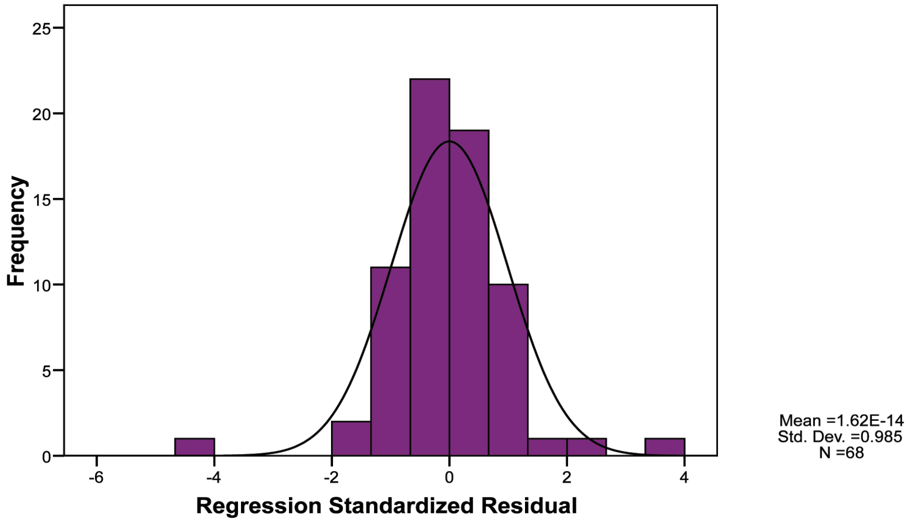


Fig.4: Histogram of the residuals data that has a bell-shaped curve which shows that residuals are normally distributed

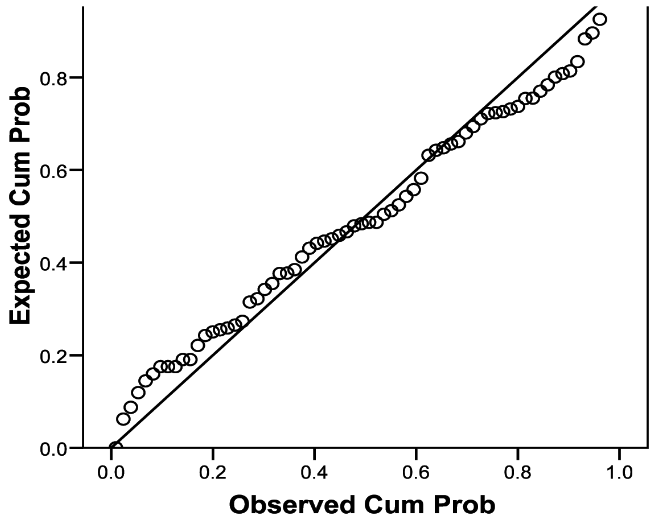


Fig.4: Normal P-P plot shows the points lie along the diagonal line indicating residuals are normally distributed

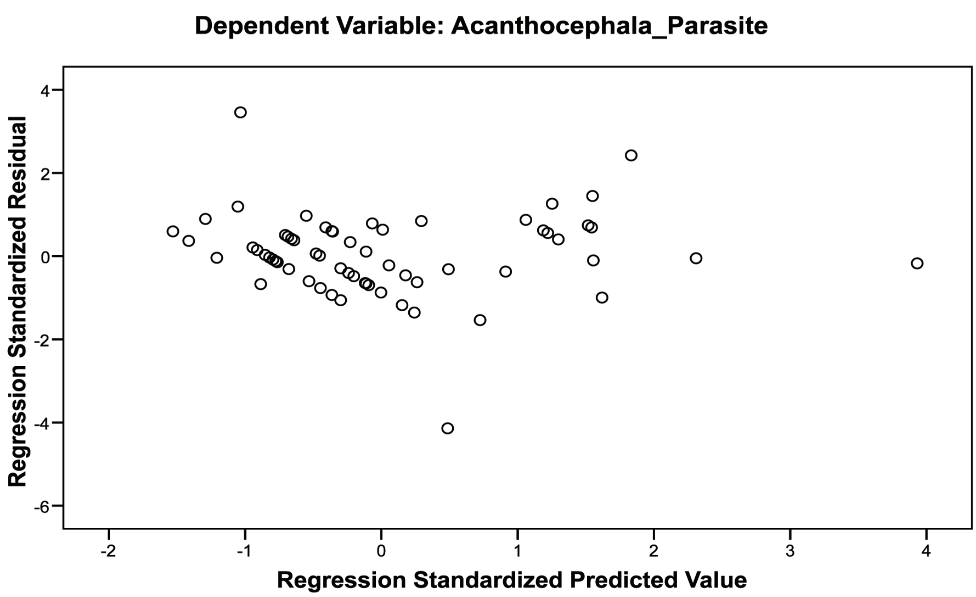


Fig.5: Points were randomly and evenly dispersed throughout the plot. Pattern shows that the assumptions of linearity and homoscedasticity of the residuals have been met

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