Journal of Global Biosciences
ISSN 2320-1355
Volume 5, Number 1, 2016, pp. 3500-3512
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

INTENSITY AND DISPERSAL OF RUMEN FLUKES OF CATTLE IN SELECTED AREAS OF SRI LANKA

Lalithanjalie Deepika Amarasinghe and Thusith Maduranga

Department of Zoology and Environmental Management,
Faculty of Science,
University of Kelaniya, Sri Lanka.

Abstract

Diversity and density of the rumen fluke species of cattle (n=12 individuals x7 areas) in Vavuniya, Anuradhapura, Puttalam, Chilaw, Kurunegala, Udawalawa and Ampara areas were examined during six months period from June 2013 to November 2013. This study revealed *Paramphistomum*sp.1, *Fischoederius elongates, Fischoederiuscobboldii, Gastrothykixcrumenifer, Carmyerius*sp.1 and *Calicophoron*sp.1in infested cattle from seven areas in Sri Lanka. *Paramphistomum*sp.1 was the highly abundant rumen fluke species among the study areas. Highest density of flukes was recorded from Chilaw area while that of lowest recorded from Ampara area. Highest evenness (J') and species diversity (H') were recorded from Chilaw area in the Intermediate zone. Lowest evenness and lowest species diversity were recorded from Anuradhapura and Vavuniya areas respectivelyin the Dry zone. Mean density of each fluke species was significantly vary between areas. There was also a significant difference between the species in the same area. Seven study areas were separated into three clusters according to the hierarchical cluster analysis based on Bray-Curtis similarity matrix. These three clusters were statistically significant in pair wise test of one-way ANOSIM.

Key words: *Bosindicus*, Bos*taurus*, amphistomes, paramphistomes.

INTRODUCTION

Gastro intestinal trematodes also known as amphistomes, stomach flukesor rumen flukes are common parasites in cattle, *Bosindicus* and *Bostaurus,* inAsia, Africa and mainly in the Indian subcontinent [1].However, parasite infections in cattle receivecomparatively little attention, partly due to lack of sufficient evidence on their pathogenicity and partly due to the facts that they are seen in post mortem and other pathological examinations more frequently[2].Though the mature stages of amphistomes live in the rumen and reticulum, their immature stages are found in the small intestine, embedding in and penetrating the gut mucosa of ruminants. This causes to bleeding and necrosis in the gut wall of infected animals. Adults cause to bleeding and localize destruction of papillae in rumen and reticulum. If present in sufficient numbers damage may be responsible for listlessness, anaemia and diarrohea in the animal and severe cases lead to death especially in young calves. Pathogenic effect associated with adult flukes is less comparing with the effect of immature ones [3].It is reported that claves are more susceptible to the effects of parasitism during the first five to eight months after being exposed to significant levels of infection [4].Deaths in cattle as a result of paramphistomeinfection,amphistomes of the

Family Paramphistomidae, have been reported from several countries. In Sri Lanka, death of a cow has reported due to the massive infection of rumen fluke, *Cotylophoron* spp.[5].The economic impact of gastrointestinal parasites results in mortality losses, reduction in growth and production and also condemnation of carcasses as well as high cost of drugs and veterinary care[6].

Some liver, blood and gastrointestinal flukes have been recorded from Sri Lanka. They are belonged to genera of *Fasciola, Schistosoma, Paramphistomum, Fischoederius, Gastrothylax, Ceylanocotyle*and*Calicophoron*[7].*Gastrothylaxcrumenifer*, *Fischoederius elongates*, *Carmyerius* spp., *Paramphistomum* spp. and *Explanatumexplanatum* have been recorded from cattle in Gampaha district in Sri Lanka [8].However, there is not much research have been carried out to investigate the distribution and abundance of trematode species throughout main cattle rearingareas in Sri Lanka.

Livestock farming, specially rearing of cattle (*Bosindicus* / *Bostaurus*), is a tradition among the rural people in Sri Lanka. Cattle have played an important role in the agricultural economy of Sri Lanka since ancient times. According to the estimations of year 2012, there are 1,235,535 number of cattle live in Sri Lanka [9].Traditionally cattle are reared as small or large herds in the free grazing lands in Sri Lanka. This method is known as the extensive cattle farming system. Most of the time these cattle are grazed on abandoned or post-harvested paddy fields, scrub lands, communal or private lands. Cattle farming by the intensive grazing on well managed grass lands also practiced in Sri Lanka [10].

The objectives of the present study are,to identify and determine the intensity and distribution of the rumen flukespecies present in cattle in main cattle rearing areas,Anuradhapura, Puttlam, Vauniya, Chilaw, Kurunegala, Udawalawa and Amparain Sri Lanka.

MATERIALS AND METHODS

Sample collection: This study was conducted using slaughtered animals brought from Vavuniya, Anuradhapura, Puttalam, Udawalawa, Ampara in the dry zone and Chilaw and Kurunegalain the intermediate zone of Sri Lanka to the meat inspection abattoir of Colombo Municipal Council, at Dematagoda during the periodfrom June 2013 to November 2013.

Collection of parasite specimens: Rumen portions were separated from carcassesand carefully examined for presence of flukesat the abattoir. About 100 g of parasite infested rumen sample wasseparated and brought intothe laboratory in labeled polythene bag. Rumen samples from twelve carcassesbrought from each areawere collected for this study.

Processing of parasite specimens: In the laboratory,live flukes were removed from individual rumen sample using a pair of forceps and collected in a metal tray containing mammalian saline. They were killed by adding 5% formal saline solution into them for about 10 minutes.Parasites were separated into groups according to their external morphological features and body shapes. Few best quality specimens from each group were selected and washed in mammalian saline twice for preparation ofwhole mountsand histological sectioning. Rest of the specimens were stored separately in 10% buffered formalin for accurate counting of number. Weight of the rumen sample was recorded after the removal of all the flukes attached to that rumen sample.

Preparation of whole mounts: Flukes were flattened individually between two glass slides and tightened the pair of glass slides using a rubber band. This was fixed overnight in 80% alcohol.Flattened specimens were removed from 80% alcohol and were hydrated using an alcohol series 50%, 30%, and 10% each for 30 minutes each. Specimens werewashed in distilled water and placed separately in watch glasses containingaceto alum carmine stain for 30minutes to two hours. Specimens were washed in 50% alcohol to remove excess stain. Over stained specimens were differentiated with acid alcohol and washed in distilled water. Then, the specimens were dehydrated using an alcohol series, 30%, 50%, 70%, and 90%, and two changes of absolute alcohol each for 30minutes. Specimens were cleared in xylene and mounted in Canada balsam.Flukes were identified using the key published by International Institute of Parasitology, ST Albans UK, [11].

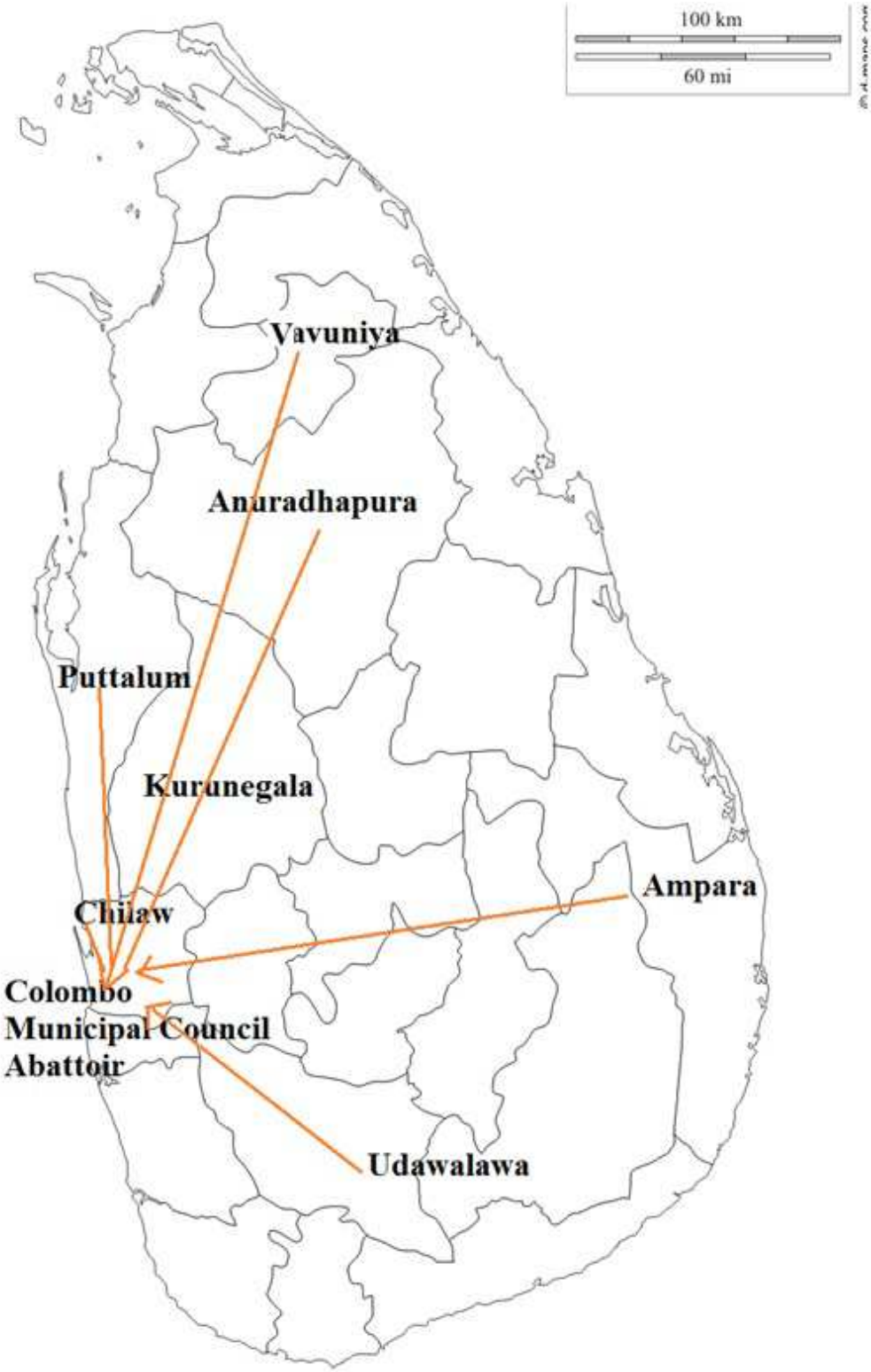

Figure 1: Map showing study areas from where the rumen samples of the slaughtered cattle were obtained.

Preparation of stained histological sections of rumen flukes: Formalin-acetic acid-alcohol fixative (FAA fixative) was heated to 60 ºC in a water bath. Washed flukes from mammalian saline were added to it and fixed for 24 hours and stored in 10% buffered formalin until further processing.Fixed specimens were dehydrated using alcohol series 30%, 50%, 70%, and 90%, and three changes of absolute alcohol each for 2 hours and stored overnight in chloroform. After

that wax infiltration was taken place in three changes of paraffin wax baths, each for one hour. Finally specimens were embedded in fresh wax blocks.About 14µm thick histological sections of the specimens were obtained using the microtome (model: Reichert-jung-2030). Sections were mounted on egg albumen coated glass slides. Sections were de-waxed in xylene for three minutes. Then they were given two changes of absolute alcohol each longing for two minutes. Specimens were transferred into 90% alcohol and then into 70% alcohol each for two minutes. Specimens were stained using Ehrlich's Haematoxaline for 30 minutes. After that they were washed in running tap water for about 2-3 minutes. Then the specimens stained with Eosin for about 15 seconds. Again they were washed in running tap water until the excess stain was removed. They were dehydrated using an alcohol series (70%, 90%, and two changes of absolute alcohol each for 2 minutes). After that sections were clear in xylene for three minutes and then mounted using Canada balsm.Flukes were identified using the key published by International Institute of Parasitology, ST Albans UK, [11].The total number collected from each genera or species in each rumen sample were separately counted.

Data Analysis Mean density of each fluke species in infested rumens in each area (number of flukes per 100 g of rumen) were analyzed using One-Way ANOVA to examine whether the mean densities of flukes in each species are significantly differentbetween seven sampling areas. The accepted level of significance was $P<0.05$. Tukey's pair wise comparison was done to examine which sampling areas were significantly different in mean density of each fluke species.Mean densities of each fluke species in each area were analyzed using One-Way ANOVA to examine whether the mean densities of fluke species in same area were significantly different from each other or not. The accepted level of significance was $P<0.05$. Tukey's pair wise comparison was done to examine density of which species are significantly different from each other. Statistical software package MINITAB®release 14, © 1972-2004 Minitab Inc was used for data analysis.

Species richness (SR), total abundance (N), Pielou's evenness index (J') and Shannon-Weiner heterogeneity index (H') were calculated for total numbers of each fluke species in each area for 1200g (100g×12 cattle) of rumen samples using PRIMER 5 software.Cluster analysis was done to the total numbers of flukes in each species in each area for 1200g of rumen sample. The cluster analysis is based on the Bray-Curtis similarity index to yield dendograms and Multi Dimensional Scaling (MDS) plots. Clusters were identified using dendogram of Bray-Curtis similarity index and Multi Dimensional Scaling (MDS) plots. One-way ANOSIM test was performed to find out whether there were significant differences between the major clusters in dendogram.Statistical software package *PRIMER 5 forWindows Version 5.2.9* © Copyright 2002 PEIMER-E Ltd was used for data analysis.

RESULTS

Identification of rumen flukes: During this study, *Paramphistomum*sp. 1, *Fischoederius elongates, Fischoederiuscobboldii*, *Gastrothylaxcrumenifer, Carmyerius*sp. 1, and *Calicophoron*sp. 1wereidentified as mixed infestationsin rumen wall.

Paramphistomumsp. 1**. (Fishoeder, 1901)**which is belonged to the Family-Paramphistomoidae was found adhering to the walls of rumen and reticulum of cattle. Their definitive hosts are*Bosindicus, Ovisaries*and *Capra hircus.* Live worms were reddish pink in colour. Body was conical in shape and maggot like. They changed into ashy white colour after killing them using 5% formal saline. They became darker in colour after fixing in formalin-acetic acid-alcohol fixative (FAA fixative). In these amphistomes body is elongated conical shape and comparatively smaller than other amphistomes. They are about 5-10 mm in length and 2-4 mm in external maximum diameter. Acetabulum is sub terminal and 1-2.5 mm in external diameter. They do not have oral sucker and ventral pouch. Mouth is at the anterior end of the body. Alimentary canal consists of pharynx, oesophagus, and pair of intestinal caeca. They do not have oesophageal bulb. Caeca in lateral fields, forming irregular bends throughout their course, reaching up to level of acetabulum. They have two testes and they are tandem and lobed. Ovary is posterior to the testes. Vitellaria extending from level of oesophagus to acetabular region, in lateral fields, sometimes encroaching in inter caecal space in acetabular region. Major identification features are the**s**hape and size of the body, absence of oral sucker and ventral

pouch, sub terminal acetabulum, arrangement and location of the testes and ovary, location within the host.

***Fischoederius elongates* (Poirier, 1883) (**Family-Gastrothylacidae)were found adhering to the walls of rumen of cattle. Their definitive hosts are*Bosindicus, Bubalusbubalis, Ovisaries*and *Capra hircus.* Live worms were dark red in colour. Body was elongated. They changed into grayish white colour after killing them using 5% formal saline. They became darker in colour after fixing in formalin-acetic acid-alcohol fixative (FAA fixative). In these amphistomes body is longer compare to other amphistomes. They are about 6-22 mm in length and 3-6 mm in external diameter. Acetabulum is terminal and 1-3 mm in diameter. Comparing with other amphistomes, it is smaller in size. They do not have oral sucker. They have ventral pouch and it has an elongated triangular shape in a median sagittal section. Mouth is at the anterior end of the body. Alimentary canal is well developed. It consists of mouth, pharynx, oesophagus, and pair of intestinal caeca. They do not have oesophageal bulb. Caeca in lateral fields, running parallel to each other and have a wavy appearance. Caeca are terminated before middle of the body. These amphistomes have two testes and they are tandem and lobed. Ovary is inter-testicular. Vitellaria are small and located in lateral fields extending from level of oesophageal bifurcation to ovary. Terminal genitalium is presented ventrally inside ventral pouch at level posterior to pharynx. Major identification features are the elongated body, absence of oral sucker and presence of ventral pouch, arrangement and location of the testes and ovary.

***Fischoederiuscobboldii*(Poirier, 1833)**Family-Gastrothylacidae were found adhering to the walls of the rumen of cattle. Their definitive hosts are*Bosindicus, Bubalusbubalis, Ovisaries*and *Capra hircus.* Live worms were bright to dark red in colour. Body was conical in shape and maggot like. They changed into ashy white colour after killing them using 5% formal saline. They became darker in colour after fixing in formalin-acetic acid-alcohol fixative (FAA fixative). In these amphistomes body is elongated. They are about 7-17 mm in length and 5-9 mm in external diameter of the body. Acetabulum is terminal and 3-7 mm in external diameter. They do not have oral sucker. Ventral pouch is present. Mouth is at the anterior end of the body. Alimentary canal consists of pharynx, oesophagus, and pair of intestinal caeca. Oesophagus is 'S' shaped. Caeca reaching up to anterior border of acetabulum. They have two testes and they are tandem, irregular and lobed. Ovary is posterior to the testes. Vitellaria sparsely scattered follicles in ventro-lateral regions, extending from level of oesophageal bifurcation to anterior margin of acetabulum. Major identification features arethe shape and size of the body, presence of comparatively larger acetabulum, absence of oral sucker, Presence of ventral pouch, arrangement and location of the testes and ovary, location within the host.

***Gastrothylaxcrumenifer*(Creplin, 1847)**Family-Gastrothylacidaewere found adhering to the walls of the rumen of cattle.Their definitive hosts are*Bosindicus, Bubalusbubalis, Ovisaries*and *Capra hircus.* Live worms were dark red in colour. Body was conical in shape and maggot like. They changed into whitish colour after killing them using 5% formal saline. They became darker in colour after fixing in formalin-acetic acid-alcohol fixative (FAA fixative). In these amphistomes body is oval shaped and comparatively larger. They are about 10-19 mm in length and 5-8 mm in width. Acetabulum is terminal and 1-5 mm in external diameter. They do not have oral sucker. They have ventral pouch. Mouth is at the anterior end of the body. Alimentary canal is well developed. It consists of pharynx, oesophagus, and pair of intestinal caeca. They do not have oesophageal bulb. Intestinal caeca are long, wavy and reaching up to anterior level of testes. They have two testes and they are tandem and deeply lobed side by side, between caecal ends and acetabulum. Ovary is inter-testicular. Male and female reproductive systems have common genitalium. Vitellaria are small, follicular and extending from intestinal fork to acetabulum. Uterus crosses from one side of the body to the other side.Major identification features are the shape and size of the body, absence of oral sucker, presence of ventral pouch, arrangement and location of the testes and ovary, arrangement of the uterus.

***Carmyerius*sp. 1. (Fischoeder, 1901)** Family-Gastrothylacidae were found adhering to the walls of the rumen of cattle.Their definitive hosts are*Bosindicus, Bubalusbubalis, Ovisaries*and *Capra hircus.* Live worms were red in colour. Body was elongated in shape. They changed into whitish colour after killing them using 5% formal saline. They became ashy white in colour after

fixing in formalin-acetic acid-alcohol fixative (FAA fixative). In these amphistomes body is elongated. They are about 7-12 mm in length and 2-5 mm in external maximum diameter. Acetabulum is terminal and 1-2 mm in external diameter. They do not have oral sucker. They have triangular shape ventral pouch. But in median sagittal sections it may be variable in shape. Mouth is at the anterior end of the body. Alimentary canal consists of pharynx, oesophagus, and pair of intestinal caeca. Caeca are straight and reaching up to posterior region of body. They do not have oesophageal bulb. They have two testes and they are lobed and symmetrical and located laterally near acetabulum. Ovary is posterior to the testes. The uterus is ascending in the mid field of the body throughout its body. Vitellaria are consisted of small follicles, scattered from level of pharynx to testicular region. Genitalium opens inside the ventral pouch. Major identification features are the shape and size of the body, shape of the ventral pouch and arrangement of the uterus.

Calicophoronsp. 1**. (Fishoeder, 1901)**Family-Paramphistomoidae were found adhering to the walls of rumen of cattle. Their definitive hosts are*Bosindicus, Ovisaries, Bubalusbubalis*and *Capra hircus.* Live worms were pinkish white colour. Body was conical in shape. They changed into ashy white colour after killing them using 5% formal saline. They became ashy white in colour after fixing in formalin-acetic acid-alcohol fixative (FAA fixative). In these amphistomes body is elongated conical shape and slightly bent ventrally. They are comparatively broader than other amphistomes. They are about 7-11 mm in length and 5-9 mm in external maximum diameter. Acetabulum is sub terminal and 1.5-2.5 mm in external diameter. They do not have oral sucker and ventral pouch. Mouth is at the anterior end of the body. Alimentary canal consists of pharynx, oesophagus, and pair of intestinal caeca. They do not have oesophageal bulb. Caeca on lateral fields, forming few irregular dorso-ventral bends. They have two testes and they are tandem and lobed. Ovary is posterior to the testes. Vitellaria in lateral fields are extended from level of pharynx to acetabulum. Major identification features are the presence of conical shape body with an anterior end that is slightly bent ventrally. Acetabulum is sub terminal. Body is light pinkish in colour.

Intensity and distribution of the rumen flukes: The mean density of digenean fluke species in 100g of rumen (n=12) of infested cattle from seven study areas of Sri Lanka are given in Table 1. According to Table 1,*Paramphistomum*sp. 1 and *Calicophoron*sp. 1were not recorded from Udawalawa and Ampara areas but all the other areas were positive for these two species.*Paramphistomum*sp. 1was highly abundant in Anuradhapura (208.09 ± 62.74SE), Puttalam (157.57 ± 37.29SE) and Vavuniya (172.55 ± 33.89SE) areas compared to that of Chilaw (96.96 ±30.05SE) and Kurunegala (57.61 ± 11.85SE) areas. *Calicophoron*sp. 1was recorded comparatively in very low densities in recorded area (Anuradhapura, Puttalam, Vavuniya, Chilaw and Kurunegala areas). *Fischoederius elongates* was recorded from Chilaw, Kurunegala, Udawalawa and Ampara areas. They were highly abundant in Udawalawa (122.95 ± 28.46SE) and Ampara (91.71 ± 22.08SE) areas.*Fischoederiuscobboldii* was recorded only from Chilaw (27.07 ± 9.82SE) and Ampara (41.52 ±13.60SE) areas and their densities were comparatively low in those areas. *Gastrothylaxcrumenifer* was recorded from Puttalam, Chilaw, Kurunegala, Udawalawa and Amparaareas. But they were not dominated in densities in these areas.*Carmyerius*sp. 1was recorded from Anuradhapura, Puttalam, Chilaw, Kurunegala, Udawalawa and Ampara areas. They could be found in higher densities in Chilaw (93.70 ± 26.53SE), Kurunegala (89.42 ± 29.98SE) and Anuradhapura (80.06 ± 24.42SE) areas. According to these data, highest level of fluke infestation could be observed in cattle lived in Chilaw area (326.69) followed by Anuradhapura area (302.12). Cattle lived in Ampara areawere shown lowest infestation (190.02) followed by Udawalawa area (198.67). Puttalam, Vavuniya and Kurunegala areas were laid in between these two extremes (247.52, 211.69 and 209.34 respectively) of which Vavuniya and Kurunegala areas have shown almost similar infestation levels.

Table 1: Mean density of digenean fluke species in 100g of rumen (n=12) of infested cattle from seven study areas of Sri Lanka

Species of flukes	Mean density ± SE						
	Anuradhapura*	Puttalam *	Vauniya *	Chilaw *	Kurunegala *	Udawalawa *	Ampara *
*Paramphistomums*p. 1 *	208.09 ± 62.74$^{a, a}$ (615.38-0.00)	157.57 ± 37.29^{a,b,} ^{a}(348.53-0.00)	172.55 ± 33.89^{a,b,} ^{a}(322.37-0.00)	96.96 ± 30.05$^{a,b,c, a}$ (301.59-0.00)	57.61 ± 11.85$^{b,c, a,b}$ (114.08-0.00)	0.00 ± 0.00$^{c, a}$	0.00 ± 0.00$^{c, a}$
Fischoederius elongates *	0.00 ± 0.00$^{a, b}$	0.00 ± 0.00$^{a, b}$	0.00 ± 0.00$^{a, b}$	69.76 ± 25.39^{a,b,} ^{a,b}(225.03-0.00)	23.27 ± 10.31^{a,c,} ^{a} (106.17-0.00)	122.95 ± 28.46$^{b, b}$ (310.95-0.00)	91.71 ± 22.08$^{b,c, b}$ (243.14-0.00)
Fischoederiuscobboldii *	0.00 ± 0.00$^{a, b}$	0.00 ± 0.00$^{a, b}$	0.00 ± 0.00$^{a, b}$	27.07 ± 9.82^{a,b,} ^{a,b}(88.40-0.00)	0.00 ± 0.00$^{a, a}$	0.00 ± 0.00$^{a, a}$	41.52 ± 13.60^{b,} ^{a}(131.43-0.00)
Gastrothylaxcrumenifer *	0.00 ± 0.00$^{a, b}$	51.73 ± 22.38^{a,} ^{b}(176.23-0.00)	0.00 ± 0.00$^{a, b}$	30.96 ± 8.81^{a,} ^{a,b} (97.26-0.00)	31.29 ± 15.02^{a,} ^{a,b}(143.16-0.00)	54.06 ± 16.61^{a,} ^{a} (168.10-0.00)	36.14 ± 12.32^{a,} ^{a}(115.49-0.00)
*Carmyeriuss*p. 1 *	80.06 ± 24.42^{a,b,} ^{b}(238.21-0.00)	24.33 ± 14.03$^{a,b, b}$ (144.74-0.00)	0.00 ± 0.00$^{a, b}$	93.70 ± 26.53^{b,} ^{a}(260.16-0.00)	89.42 ± 29.98^{b,} ^{b}(268.79-0.00)	21.67 ± 5.78$^{a,b, a}$ (48.05-0.00)	20.66 ± 5.50^{a,b,} ^{a}(44.88-0.00)
*Calicophorons*p. 1 *	13.97 ± 7.32^{a,} ^{b}(61.27-0.00)	13.88 ± 6.32^{a,} ^{b}(60.50-0.00)	39.14 ± 9.61$^{b, b}$ (94.83-0.00)	8.24 ± 4.69$^{a, b}$ (50.52-0.00)	7.75 ± 3.61$^{a, a}$ (34.93-0.00)	0.00 ± 0.00$^{a, a}$	0.00 ± 0.00$^{a, a}$
Total of means	302.12	247.52	211.69	326.69	209.34	198.67	190.02

Note: Values represent Mean ± SE and range in parenthesis. '*' indicates significant P – values detected from One-Way ANOVA. Different black colour superscript lettersin a row show significant differences (P<0.05) between areas for each species and different bold superscript letters in a column show significant difference (P<0.05) between species for each area indicated by Tukey's pair-wise comparisons after One-Way ANOVA.

One-Way ANOVA revealed thatthe mean density of each fluke species between areas were significantly different (df=6, P<0.05) (Table 1), *Paramphistomum*sp. 1(f=6.53, P=0.000); *Fischoederius elongates* (f=8.77, P=0.000); *Fischoederiuscobboldii* (f=7.40, P=0.000); *Gastrothylaxcrumenifer* (f=2.73, P=0.018); *Carmyerius*sp. 1 (f=4.33, P=0.001) and *Calicophoron*sp. 1(f=5.62, P=0.000) respectively. According to the Tukey's pair-wise comparison (Table 1) there was always a significant difference in the density of each fluke species except *Gastrothylaxcrumenifer* at least between two areas (P<0.05). According to this comparison there were significant differences of the densities of *Paramphistomum*sp. 1between Anuradhapura (208.09 ± 62.74SE) and Kurunegala (57.61 ± 11.85SE) areas anddensities of the *Fischoederius elongates* between Kurunegala (23.27 ± 10.31SE) and Udawalawa (122.95 ± 28.46SE) areas.There was no significant difference of the densities of *Fischoederiuscobboldii*betweenAmpara (41.52 ± 13.60SE) andChilaw (27.07 ± 9.82SE) area. There was no significant difference among the recorded areas for the densities of*Gastrothylaxcrumenifer*. The densities of *Carmyerius*sp. 1among the recorded areas were not significantly different. The densities of the *Calicophoron*sp. 1inVavuniya (39.14 ± 9.61SE) areawas significantly different from Anuradhapura (13.97 ± 7.32SE), Puttalam (13.88 ± 6.32SE), Chilaw (8.24 ± 4.69SE) and Kurunegala (7.75 ± 3.61SE) areas.One-Way ANOVA revealed that there were significant differences of the densities of fluke species in each area (df=6, P<0.05) (Table 1), Anuradhapura (f=9.08, P=0.000); Puttalam (f=10.20, P=0.000); Vavuniya (f=23.05, P=0.000); Chilaw (f=3.44, P=0.008); Kurunegala (f=4.85, P=0.001); Udawalawa (f=12.79, P=0.000) and Ampara (f=8.21, P=0.000) respectively. According to the Tukey's pair-wise comparison (Table 1) has shown that there was always a significant difference between the density of fluke speciesin same area (P<0.05). The mean densities of *Paramphistomum* sp. 1 in Anuradhapura (208.09 ± 62.74SE), Puttalam (157.57 ± 37.29SE) and Vauniya (172.55 ± 33.89SE) areas were significantly different from other species recorded from those three areas. That means according to the Tukey's pair-wise comparison, a similarity of the density distribution can be identified among Anuradhapura, Puttalam and Vavuniya areas. The mean densities of *Fischoederius elongates* in Udawalawa (122.95 ± 28.46SE) and Ampara (91.71 ± 22.08SE) areas were significantly different from other fluke species recorded from these two areas. That means according to the Tukey's pair-wise comparison, a similarity of the density distribution can be identified between Udawalawa and Ampara areas. The mean densities of *Paramphistomum*sp. 1(96.96 ± 30.05SE) and *Carmyerius*sp. 1(93.70 ± 26.53SE) in Chilaw area were significantly different from the mean density of *Calicophoron*sp. 1.Mean density of *Carmyerius*sp. 1(89.42 ± 29.98SE) was significantly different from the mean densities of *Calicophoron*sp. 1(7.75 ± 3.61SE) and *F. elongates*(23.27 ±10.31SE) in Kurunegala area.

Table 2: Variation of the diversity of digenean flukes of the rumen of cattle at the seven study areas in Sri Lanka

Area	Species Richness (SR)	Total Abundance (N)	Pielou's evenness index (J')	Shannon-Wiener Heterogeneity Index (H')
Anuradhapura	3	3625	0.6835	0.7509
Puttalam	4	2970	0.7244	1.0040
Vavuniya	2	2540	0.6906	0.4787
Chilaw	6	3920	0.8767	1.5710
Kurunegala	5	2512	0.8504	1.3690
Udawalawa	3	2384	0.8127	0.8928
Ampara	4	2280	0.8951	1.2410

According to the Table 2, the highest species richness and total abundance were recorded from Chilaw area. The lowest species richness value was recorded from Vavuniya area and lowest total abundance was recorded from Ampara area. According to the Pielou's evenness index, the highest evenness among the species was obtained from Ampara area followed by Chilaw area and lowest evenness among the species was obtained from Anuradhapura area followed by Vavuniya area. Species evenness of Puttalam, Kurunegala and Udawalawa areas were laid in between these two extremes.According to the Shannon-Weiner heterogeneity index, highest species diversity was obtained from Chilaw area followed by Kurunegala area. Lowest species diversity was obtained from Vavuniya area followed by Anuradhapura area. Species diversity of Ampara, Udawalawa and Puttalam areas were laid in between these two extremes.

Figure 2 shows that hierarchical cluster analysis based on Bray-Curtis similarity matrix of the total number of flukes from each species of seven areas. It was indicated that Chilaw and Kurunegala areas were clustered at 88.74% of similarity level while Udawalawa and Ampara areas were clustered at 84.25% of similarity level. Puttalamand Anuradhapura areas were clustered at 82.27% of similarity level. It also indicated that Chilaw, Kurunegala, Puttalam and Anuradhapura areas were clustered together at 74.07% of similarity level. Vavuniya area was separated from this cluster at 59.63% of similarity level. This data set produced three clusters at about 60% of similarity level (Figure 2).

Multi-dimensional scaling (MDS) plot was displayed these identified three clusters (Figure 3). R values obtained from the one-way ANOSIM test were used for the pair wise comparison of the clusters. R value usually falls between 0 and 1, indicating some degree of discrimination between the sites. R value is equal to one only if all replicates within sites are more similar to each other than any replicates from different sites. R value is approximately zero if the similarities between and within sites will be the same on average (Clarke and Goorley, 2001). According to the pair wise comparison test, these three clusters were significantly different from each other (Cluster A-B, R=1.000 / Cluster A-C, R=0.857 / Cluster B-C, R=0.667) ($P<0.05$, one-way ANOSIM).

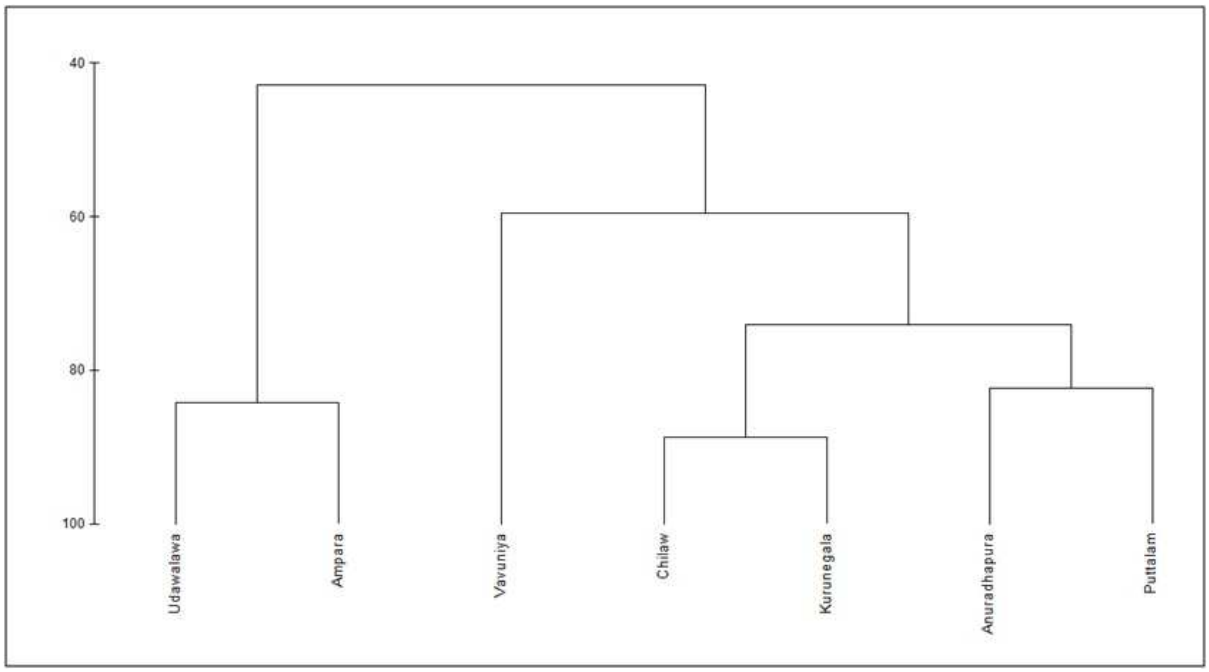

Figure2:Dendrogram of Bray-Curtis similarity matrix for the transformed total numbers of flukes in each species in each area for observed 12 rumen samples. The data set produced three clusters at 59.63 similarity level.

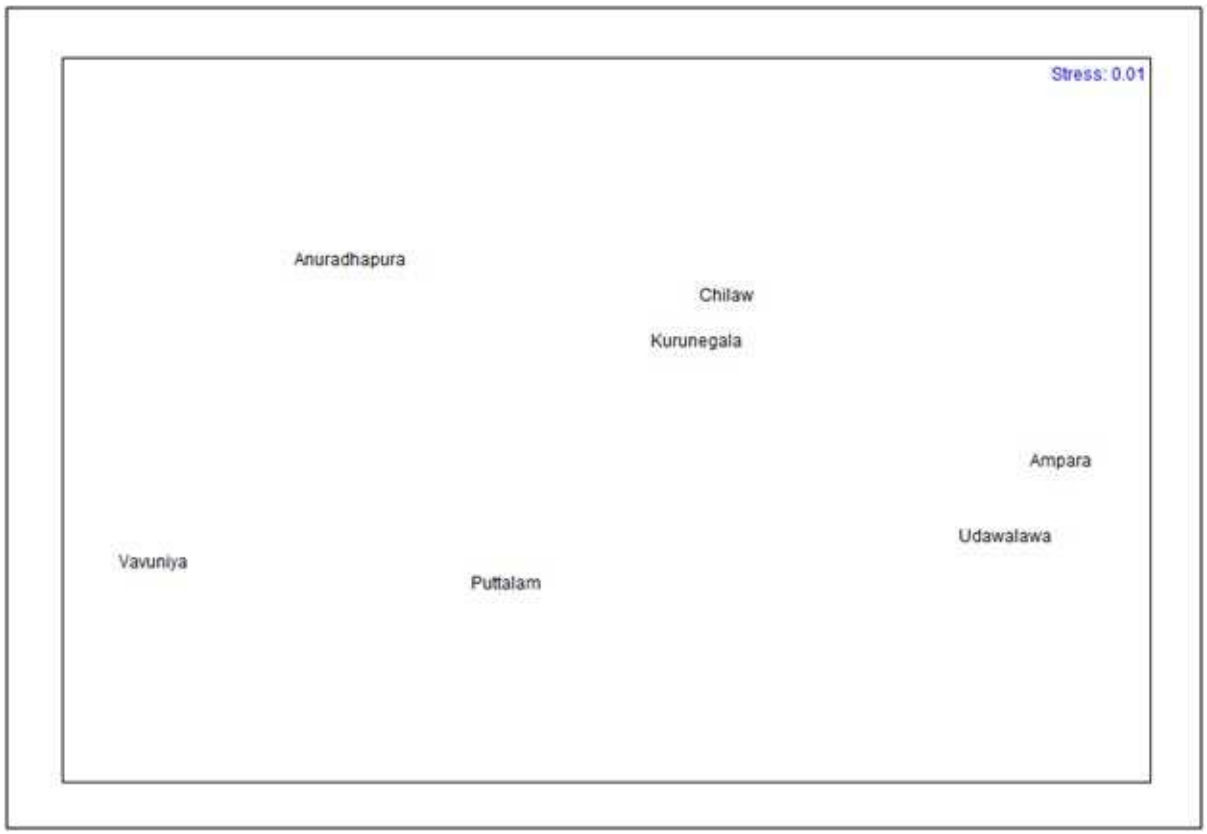

Figure3: Multi-dimensional scaling (MDS) plot for the transformed total numbers of flukes in each species in each area for observed 12 rumen samples. The three clusters those were identified from the dendrogram of Bray-Curtis similarity (Figure 2) are indicated here. The MDS ordination gave a stress value of 0.01 indicating that two-dimensional ordination is sufficient (Clarke and Gorley, 2001).

DISCUSSION

During this study, six digeneantrematode species were found in rumens of the slaughtered cattle in selected cattle rearing areas. Whole mounts and histological sections were very much useful to identify these species because most of the internal organs and their arrangements were clearly seen. The identified digeneans were *Paramphistomum*sp. 1, *Fischoederius elongates*, *Fischoederiuscobboldii*, *Gastrothylaxcrumenifer*, *Carmyerius*sp. 1and *Calicophoron*sp. 1. This observation compiles with the past records [7, 8,12]. *Carmyerius*sp. 1was first recorded in cattle of Ja-Ela area in Gampaha district in 2007 [8]. Present study revealed that *Carmyerius*sp. 1has extensively distributed within the country. *F. cobboldii*and *Calicophoron*sp. 1were reported as wide spread common parasitesin the rumen of cattle and buffaloes in the dry zone areas in the year 1955 [13]. In this study, *Ceylanocotyles*spp.was not recorded from any study area. However, this species was recorded from a higher elevation of the country in Kandy area in a previous survey [13].

Highest infestation of rumen flukes was observed from the samples collected fromChilaw area followed by Anuradhapura area. The intensity of the parasite burden is highly depended on the prevalence of the aquaticsnails, as intermediate hosts in the grazing lands of cattle. According to this study Vavuniya and Kurunegala areas have shown almost similar infestation levels. Decision making about the fluke infestations without having reliable information regarding the distribution pattern of the aquatic snail species in these study areas is problematic. The distribution of freshwater snails accounts for the occurrence of different trematode taxa in a particular area [14].Therefore investigation of the distribution pattern of the aquatic snail species in these areas is very important.

According to the species distribution pattern (Table 1), *Paramphistomum*sp. 1was significantly highercompared to other parasite species in Anuradhapura, Puttalam and Vavuniya areas. Comparatively low numbers of*Paramphistomum*sp. 1were reported fromChilaw and Kurunegala areas. Wet zone is reported to have the highest species diversity of aquatic snailswhich is followed by Intermediate zone[15]. The highest Shannon-Weiner diversity index was obtained for Chilaw area followed by Kurunegala area. Pielou's evenness index in these two areas is also comparatively higher. This higher evenness and higher diversity reveal that there is no any domination of one species among others inChilaw and Kurunegala areas.

Fischoederius elongates was recorded from Chilaw, Kurunegala, Udawalawa and Ampara areas. They were not recorded from Anuradhapura, Puttalam and Vavuniya areas. *F. elongates* in Udawalawa and Ampara areas were significant different compared to other species. During this study *Fischoederiuscobboldii*has recorded only from Ampara and Chilaw area where higher density was recorded from Ampara. *Gastrothylaxcrumenifer*was recorded from Puttalam, Chilaw, Kurunegala, Udawalawa and Ampara areas. There is no significant different in densities of this parasite between areas. Similar observation has obtained for *Carmyerius*sp. 1where there is no significant difference between areas. The density of *Calicophoron*sp. 1in Vavuniya area has shown a significant different compared to other areas. *Paramphistomum* spp. is the only other fluke species found fromVavuniya area. Lower species richness and lower species diversity in Vavuniya area provide good vacant niches for *Calicophoron*sp. 1and therefore the density of this species may have been significantly increased.

According to the dendrogram of Bray-Curtis similarity matrix (Figure 1) and multi-dimensional scaling plot (Figure 2), three clusters were formed and they were significant different. According to the dendrogram, key factor for the separation of Vavuniya, Chilaw, Kurunegala, Anuradhapura and Puttalam areas at about 43% of similarity level is the density of *Paramphistomum* spp. and the presence of *Calicophoron*sp. 1. Udawalawa and Ampara areas were separated from this cluster due to the absence of these two parasite species. Anuradhapura, Puttalam, Chilaw and Kurunegala areas were positive for *Carmyerius*sp. 1whileVavuniya area was negative. Due to this reason Vavuniya was separated from major cluster at 59.63% of similarity level and finally three clusters were appeared at this similarity level.

According to the one-way ANOSIM test these three clusters were significantly different from each other. R value for the comparison of cluster A (Udawalawa and Ampara) with cluster B

(Vavuniya) was equal to one. That means all replicates within sites are more similar to each other than other site. This was due to absence of *Paramphistomum*sp. 1and *Calicophoron*sp. 1in Udawalawa and Ampara areas and absence of *F. elongates*, *G. crumenifer* and *Carmyerius*sp. 1in Vavuniya area. Species distribution among these two sites was completely different. According to Clarke and Gorley (2001) the interpreted R value greater than 0.75 consider as well separated cluster and R value greater than 0.5 consider as overlapping clusters but clearly different[16]. R value for the comparison of cluster A with cluster C (Chilaw, Kurunegala, Anuradhapura and Puttalam) was 0.857. So these two clusters can be considered as well separated clusters. *Paramphistomum*sp. 1and *Calicophoron*sp. 1were recorded from the areas belong to cluster C.*Carmyerius*sp. 1, *G. crumenifer,F. elongates* and *F. cobboldii* are common to both clusters. Distribution of common species among two clusters caused to this kind of observation. R value for the comparison of cluster B with cluster C was 0.667. So these two clusters can be consider as overlapping clusters but clearly different. Reason for the overlapping of the cluster B and C was the presence of*Paramphistomum*sp. 1and *Calicophoron*sp. 1in both clusters.

If the cattle population of the country was not disturbed, then a clear view about the distribution and the density of the rumen flukes could be obtained. Due to the anthropogenic activities such as legal or illegal cattle transportation within the country, the natural distribution of the cattle population has disturbed. More frequently cattle transportation take place towards the capitaldue to higher human population and higher consumption of beef. Therefore transportation of cattle is unidirectional. This may be another reason for having comparatively low number of rumen fluke species and their densities in fardistant regions while higher densities of rumen fluke species in closer areas to the capital.

REFERENCES

1. Boray, J. C., 1981. Recent advances in research on faciolasis and other trematodes infections. *Reviewed advances in parasitology* 10: 317-319.
2. Dewan, M. L., 1979. Pathological investigation on the mortality of buffalo calves of Bangladesh. *Bangladesh Veterinary Journal* 13(12): 1-7.
3. Hansen, J. and Perry, B., 1994. The epidemiology, diagnosis and control of helminth parasites of ruminents. International laboratory for research on animal diseases, Nairobi, Kenya. 171.
4. Copeman, D. B., 1982. The importance of helminth parasites in animal production systems in the tropics. In: Animal Production and Health in the Tropics. (Eds: Jainudeen, M. R. and Omar, A. R.), Chopmen Publishers, Singapore. 91-97.
5. Dawes, B., 1973. Advancess in Parasitology, 9th edition, Academic Press Inc. London. 34-35.
6. Herlich, R., 1978. Common gastrointestinal parasites of cattle, *Journal of Veterinary Science*. 10: 25-32.
7. Senadhira, M. P. A., 1967. The parasites of Ceylon (II) Trematoda. *Ceylon Veterinary Journal* 15(2): 33-41.
8. Amarasinghe, L. D. & Kumara, H.L.N.N. (2007) Effect of rainfall and temperature on liver andrumen fluke infestations of bovines in Sri Lanka. *International Journal of Biological and Chemical Sciences* 1(3): 229-236.
9. Cow and Buffalo Milk Production 1998 – 2012, Agriculture and Environment Statistics Division,Department of Census and Statistics, Sri Lanka, downloaded from http://www.statistics.gov.lk/agriculture/Livestock /MilkProduction.html on 21st of November 2013.
10. Radostitis, O. M., Blood, D. C. and Gay, C. C., 1994. Veterinary Medicine: a text book of the diseases of cattle, sheep, pigs, goats and horses. BaillereTinall Publishers, London. 1763.
11. International Institute of Parasitology, 1996. Helminthology Manual: International training course on identification of helminth parasites of economic importance, St Albans, Herts, UK.

12. Faisal, A. C. M., 1995. Helminthiasis in livestock and poultry in Sri Lanka: a Review of literature. *Sri Lanka Veterinary Journal* 42(2): 1-8.
13. Senevirathna, P., 1955. A check list of helminthes in the department of veterinary pathology, University of Ceylon, Peradeniya. *Ceylon Veterinary Journal* 15(1): 32-33.
14. Hechinger, R. F. and Lafferty, K. D., 2005. Trematodes in snail intermediate hosts. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. *Proceedings of the Royal Society* 272: 1059-1066.
15. Jayawardena, U. A., Rajakaruna, R. S. and Amarasinghe, P. H., 2010. Cercariae of trematodes in freshwater snails in three climatic zones in Sri Lanka. *Ceylon Journal of Science (Bio. Science).* 39(2): 95-108.
16. Clarke, K. R. and Gorley, R. N., 2001. PRIMER V5: User Manual/Tutorial. *PRIMER-E*, Plymouth. 1-65.