Proportion of CD4 cells before and after antiretroviral therapy (ART) in people living with HIV/AIDS visiting ART center in Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal

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Abstract

The present study deals with effect of antiretroviral treatment (ART) and effect of antiretroviral drugs (ARV) on clients attending the ART and Voluntary counseling and testing center (VCT). It is a prospective cross-sectional record-based study. According to the present study, 78 clients have taken ART age up to 54 years. Out of 78 positives, 45 (57.69%) were males and 33 (42.30%) were females. The result showed no significant relationship between age and gender (χ^2=4.196, P>0.05) of HIV positives taking ART. Majority of the positives taking ART have CD4 count less than 350 cells/mm^3 of blood and no significant relation was found between the number of positives taking ART and the CD4 cell count (χ^2=40, P>0.05). Majority of the positives i.e. 38 (48.7%) were on the WHO stage II. Majority of the positives i.e. 55 (70.5%) were given ART regimen containing Zidovudine (ZDV), Lamivudine (3TC) and Nevirapine (NVP).

The mean rise of CD4 cells i.e. 368.79 cells/µl of blood after 6 months of ART was found significant to initial CD4 count (P<0.05). Hence significant relationship was established between the ART and the increase in CD4 count (V= 8.046 P<0.05). It is also found that there is a significant increase in CD4 counts in both NVP and EFV group in unit increase of time (i.e. 6 months) (p<0.05). Also there was a significant increase (P<0.05) in weight of the positives after 6 months of ART.

***Key words**: VCT, ARV drugs, CD4 count*

Introduction

According to the report on acquired immunodeficiency syndrome (AIDS) released by the UNAIDS and World Health Organization (WHO), approximately 34 million people are living with HIV/AIDS worldwide (WHO/UNAIDS Epidemic Update 2011). The reported HIV cases in Nepal are 20,583. Out of these cases 13,157 are males while 7,417 are females and 9 are third genders. The age group between 30-39 has the highest prevalence of HIV/AIDS with 7,963 cases. Out of the total sub groups, clients of sex workers have the highest prevalence of HIV/AIDS with 8,772 cases (NCASC 2012).

Anti-retroviral therapy (ART) started in Nepal from February 2004 from Teku Hospital. Government is providing free of cost ART service to all those in need. There are National ART Guidelines and standard operating procedures for the clinical management of ART. Currently there are 39 ART centers in 33 districts in Nepal. CD4 count service is available at 16 sites, while 4 sites have CD4 caliber (NCASC 2012).

Counseling for HIV and AIDS has become a core element of a holistic model of health care; in this model, psychological issues are recognized as integral to positive management. Both pre and post counseling have become standard components of prevention-oriented HIV antibody testing programs (Valdiserri et al. 1993). The Voluntary Counseling and Testing Center (VCTC) provide a key entry point for the continuum of care in HIV/AIDS for all segments of the population.

The data collected in the present study is from ART center of Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal, may provide important clues regarding the effect of HIV positive individuals. Hence the present work is taken up to study the proportion of CD4 cells on the general public visiting ART center of Sukraraj Tropical and Infectious Disease Hospital.

Materials and Methods

The present study was conducted at the ART center of the Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal. It is a separate department of the hospital which is funded by National Center for AIDS and STD Control (NCASC) and The Global Fund under health ministry.

To investigate this study, permission from Sukraraj Tropical and Infectious Disease Hospital (STIDH), Teku, Kathmandu, authorities of Voluntary Counseling and Testing Center (VCTC) and ART center was obtained. The collected data was provided by ART center of STIDH so the reliability of the data is very high.

In the present study, only the data of positives who tested positive for HIV at the VCTC was included. This information was recorded when the positive visited the VCTC for the first time and the positives with low CD4 counts were sent to ART center for treatment. Data was collected and analyzed using the statistical package Microsoft Excel, SPSS 16 and R(R console 2.15.2). Questionnaire survey was done to know the social status of HIV positives.

Results

Demographic profile of PLHIV who are under ART

A cross-sectional study was carried out in 78 positives, undergoing ART in ART center, Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal between February 2012 to August 2012. The present study enrolled 78 PLHIV.

Out of 78 respondents, 45 (57.69%) were male while 33 (42.30%) were female. Respondent's age ranged from 4-60 years. Majority of positives were in the age group of 21-40 years (78.2%) (Table 1). No significant relationship between age and gender (χ^2=4.196, P>0.05) of HIV positives taking ART was found.

Table 1. Distribution of PLHIV taking ART according to Age and Gender

Age group	Male		Female		Total	
	No.	%	No.	%	No.	%
0-20	2	4.4	3	9.1	5	6.4
21-40	33	73.4	28	84.8	61	78.2
41-60	10	22.2	2	6.1	12	15.4
Total	45	100	33	100	78	100

(χ^2=4.196, P>0.05)

Distribution of PLHIV by Baseline CD4 count

After the positives have been counseled for ART, their social and support structure were assessed. Positives were referred to do CD4 count at every six months and monitoring of the progress was done. Follow up was maintained as: every two weeks in the first month, monthly up to the third month after the start of ART, then once in every three months and as necessary of the positives. As shown in the Table 2, about 13 of the positives (16.7%) had CD4 count<50 cells/mm^3 of blood. 9 positives (11.5%) had CD4 count between 51 to 100 cells/mm^3, 11 positives (14.1%) had CD4 count between 101 to 150 cells/mm^3, 13 positives (16.7%) had CD4 count between 151 to 200 cells/mm^3, 8 positives (10.3%) had CD4 count between 201 to 250 cells/mm^3, 8 positives (10.3%) had CD4 count between 251 to 300 cells/mm^3 , 10 positives (12.8%) had CD4 count between 300-350 cells/mm^3 and 6 positives (7.7%) had CD4 count more than 350 cells/mm^3.

Table 2. Distribution of PLHIV by Baseline CD4 count

CD4 range/cu mm blood	Male	Female	Total	%
0-50	9	4	13	16.7
51-100	4	5	9	11.5
101-150	9	2	11	14.1
151-200	7	6	13	16.7
201-250	6	2	8	10.25
251-300	2	6	8	10.25
300-350	7	3	10	12.8
350+	1	5	6	7.7
Total	45	33	78	100.0

Out of 78 positives taking ART 72 positives (92.31%) have CD4 cell count less than 350 cells/mm^3 of blood. Statistically no significant relation between the number of positives taking ART and the CD4 cell count was found (χ^2=40, P>0.05).

Distribution of the positives on the basis of WHO staging

Out of 78 positives, 10 positives (12.8%) were on WHO clinical stage I, 38 positives (48.7%) were on WHO clinical stage II, 21 positives (26.9%) were on WHO clinical stage III and 8 positives (11.5%) were on WHO clinical stage IV (Table 3).

Table 3. Distribution of PLHIV on the basis of WHO staging

WHO staging	number of positives	%
I	10	12.8
II	38	48.7
III	21	26.9
IV	9	11.5
Total	78	100

Effect of ARV drugs in PLHIV who are under ART

Antiretroviral therapy was started in all 78 positives. 56 (71.8%) positives were on the first line Nevirapine (NVP) containing regimen and 22 (28.2%) positives were on the Efavirenz (EFV) containing regimen. 55 positives (70.5%) were given ART regimen containing Zidovudine (ZDV), Lamivudine (3TC), Nevirapine (NVP); 22 positives (28.2%) were given ART regimen containing Zidovudine (ZDV), Lamivudine (3TC), Efavirenz (EFV); and 1 positive (1.3%) was given ART regimen containing Stavudine (d4T), Lamivudine (3TC), Nevirapine (NVP) (Table 4).

Table 4: ART regimen prescribed

Regimen	Frequency (number of positives)	Percentage (%)
Zidovudine+Lamivudine+Nevirapine	55	70.5
Zidovudine+Lamivudine+Efavirenz	22	28.2
Stavudine+Lamivudine+Nevirapine	1	1.3
Total	78	100.0

As shown in the Figure 1, there is a significant increase in CD4 counts in both NVP and EFV group in unit increase of time (i.e. 6 months) ($p<0.05$).

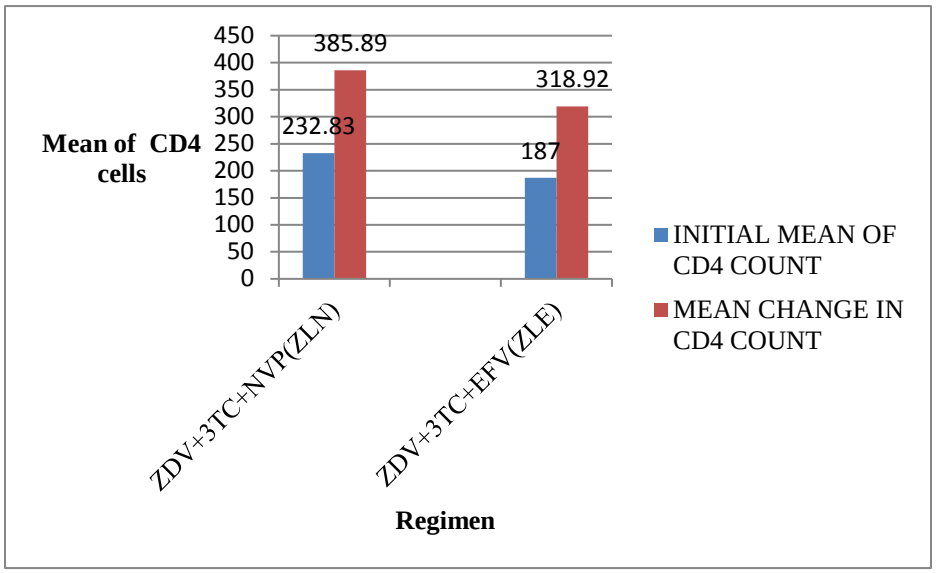


Figure 1: Initial and follow up CD4 counts

Out of the total 78 positives taken under study 47 (60.26%) were undergoing active ART. During the study period 6 positives (7.69%) lost follow up (LFU), 20 positives (25.64%) were transferred to other ART centers and 5 positives (6.41%) died.

Among 47 positives who were undergoing active ART, 43 positives (91.49%) showed normal activity throughout the study period with increase in the CD4 cells (they belong to the group with CD4 count improvement), while 4 positives (8.51%) showed decrease in CD4 count (Table 5).

Table 5. Performance of the PLHIV under ART

Features	Number (%)	Features	Number (%)
Active cases	47(60.26)	Increase in CD4 count after start of ART	43(91.49)
Lost follow up	6(7.69)	Decrease in CD4 count	4(8.51)
Transferred out	20(25.64)	No change of CD4 (stable)	0(0)
Death	5(6.41)		
Total	78(100)	Total	47(100)

Profile of CD4 counts in PLHIV who are under ART

Among 78 positives, all the positives had done CD4 count at the start but out of total samples to be studied 6 positives (7.69%) lost follow up (LFU), 20 positives (25.64%) were transferred to other ART centers and 5 positives (6.41%) died. So that only the brief ART profile of 47 active samples was available. Among the positives who had done CD4 count after six months of the start of the ART, significant relationship was found to be established between the ART and the increase in CD4 count ($V= 8.046$, $P<0.05$).

As shown in the Table 6, the mean CD4 before the start of ART is 223.21 cells/µl of blood while the mean CD4 cells after the start of ART increased to 368.79 cells/µl of blood. The minimum cell before the start of ART is 12 cells/µl of blood and the maximum cell before the start of ART is 1134 cells/µl of blood (median cells is 197 cells/µl of blood) while the minimum cell after the start of ART is 102 cells/µl of blood and the maximum cell after the start of ART is 1159 cells/µl of blood (cells/µl of blood).

Table 6. Status of CD4 counts in PLHIV

CD4 count	Frequency (no. of positives)	Mean CD4 (cells/µl)	SD	SEM	Minimum (cells/µl of blood)	Maximum (cells/µl of blood)	Median (cells/µl of blood)
Initial	47	223.21	185.98	27.1	12	1134	197
6 months	47	368.79	227.39	33.2	102	1159	370

Antiretroviral therapy and its effect on CD4 count and weight

In the study, out of 78 cases, antiretroviral therapy (ART) was given to all positives but only 47 active samples (6 lost follow up, 20 transferred out and 5 died) have active ART with prophylaxis for opportunistic infections. On comparison of pre and post ART data for CD4 count and initial and follow-up weight, there was a significant improvement in the two parameters (Table 7, Figure 2 and 3). In many cases quality of life had been improved after taking ART for six months on revealed by gaining CD4 cells and weight and their ability to do routine work actively.

Table 7. Change in CD4 count and Weight of the positive among those who are on ART Therapy during follow up

S.N.	Variable	No. on ART	Baseline value (mean±SEM)	Follow-up value (mean±SEM)	Statistical significance (wilcoxon test)	
					V value	P value
1.	CD4 count	N=47	223.21±27.1	368.79±33.2	41	P<0.001**
2.	Weight in Kg	N=47	52.98±2.07	54.17±2.12	164	P<0.05*

*significant ** highly significant

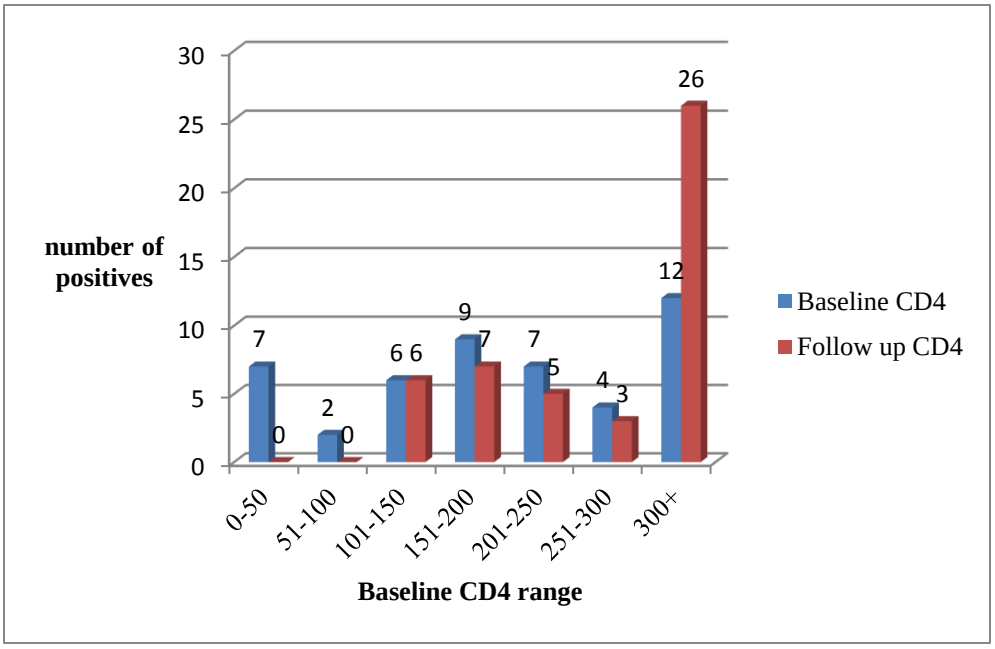
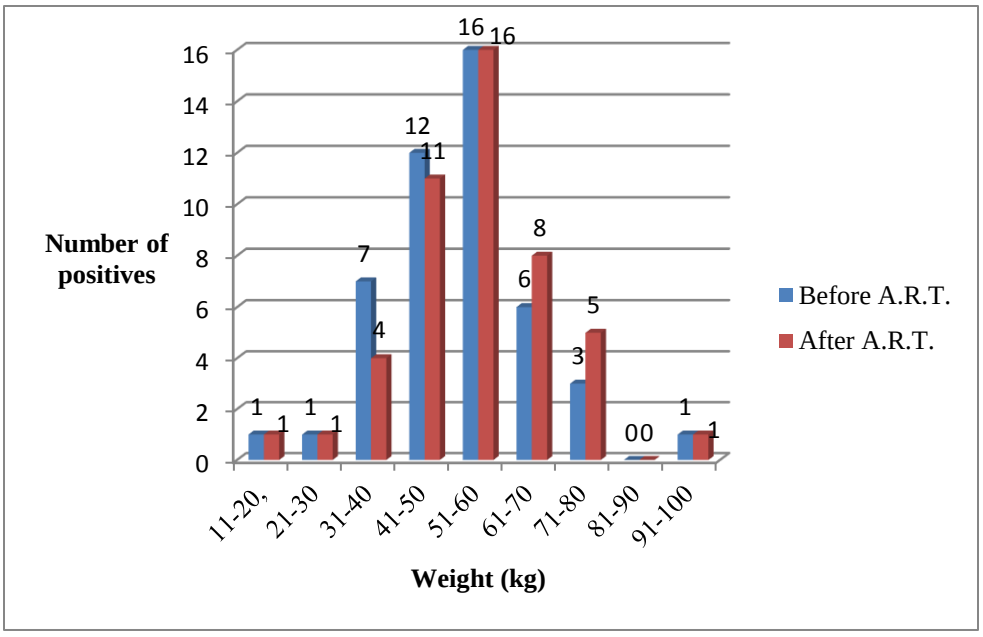


Figure 2: Distribution of PLHIV basing on Baseline and Follow up CD4 cell count



. **Figure 3**: Distribution of PLHIV basing on baseline and follow up weight in Kg

Discussion

As per the NSASC national guidelines, currently in Nepal, absolute CD4 cell count is being used as the basis for initiation of ART (NCASC 2012). In the present study, baseline mean CD4 cell count was 223.21±185.98 cells/µl of blood which is higher than the studies among similar positive groups from Nepal (Tiwari et al. 2008 and Dhakal and Aryal 2012). But Gautam et al. 2008 reported a much lower CD4 cell count of 112.1±60.29 cells/µl of blood which is much lower than the present study. CDCP has explained the AIDS surveillance case definition to include all HIV infected persons with CD4 + T- lymphocyte counts of less than 200 cells/µl of blood or a CD+ percentage of less than 14 (CDCP 1992). In the present study 56.41% cases with CD4 count less than 200 cells/µl was reported which is lower than the percentage (89.2%) reported by Gautam et al. 2008. In my study 57.69% were females which are lower than the percentage (69.29%) reported by Woldemedhin and Wabe (2012). The age group 21-30 years was predominant followed by 31-40 as reported by Sharma et al. (2009) which is similar to the present study. Sunita et al. (2011) reported in India that the majority of the positives taking ART have CD4 cell count between 50-250 cells/µl which is similar to the present study. In a study from the US, it is said that the CD4 absolute count is the best predictor of an adverse event when the CD4 count is less than 200 cells/µl while CD+ percentage is a better predictor when CD4 count is above 200 cells/µl (Pirzada et al. 2006).

The antiretroviral drug Zidovudine was introduced in 1986 for the treatment of HIV/AIDS (NCASC). Over the next few years, also other antiretroviral drugs such as nucleoside reverse transcriptase (NRTIs), non-nucleoside reverse transcriptase (NNRTIs) and protease inhibitors (PIs) will be introduced. And at present, three or more ART drugs are recommended worldwide for the treatment of HIV+ (CDCP 2002).

In this study, the mean CD4 cell count in positives at first visit to the ART center was 223.21 cells/µl of blood which increased significantly to 368.79 cells/µl of blood after six months of follow up. This finding indicated that the treatment was effective. In a study conducted by Tiwari et al. (2008) the mean CD4 cell count in the positives at first visit to Nepal Public Health Laboratory (NPHL) was 155 cells/µl of blood which increased to 297 cells/µl of blood significantly after 6 months of ART. The baseline value was quite lower than the present study and also the follow up value was lower than this study. In a study conducted by

Sunita et al. (2011) in India and Wright et al. (2011) in Australia showed that mean CD4 counts increased to above 500 cells/µl which is much higher than the present study.

Paudel et al. (2009) reported that 1 out of 53 positives (1.8%) showed decrease in CD4 cell count even after taking ART but in the present study 4 out of 47 positives (8.51%) showed decrease in CD4 cell count even after taking ART. Moore and Keruly (2007) reported 92% of the positives having an increment in the CD4 cell count which is similar with the present study (91.49%).

In the present study both NVP and EFV had similar rise in CD4 cell count from baseline and at any given point of time there was no significant difference in the rate of increase of CD4 count between the two treatments ($P<0.05$) as reported by Sunita et al. (2011). The most common 1st line regimen in this study was Zidovudine/Lamuvudine/Nevirapine which is totally different than the study done by Woldemedhin and Wabe (2012) who reported the 1st line regimen to be Stavudine/Lamivudine/Nevirapine which was similar to the study done by Kumarasamy et al. (2006).

The mean baseline weight in the study was 52.98±2.07 kg which increased to 54.17±2.12 kg which is little higher than reported by Sunita et al. (2011) who showed the baseline weight 48.7±1.0 and increment to 52.8±1.1 kg after six months of follow up of ART.

People have begun using VCTC services, which reflects a change in their attitude towards HIV. The study provides us a clue to formulate an effective approach to educate people as well as health personnel who are thought of as one of the important sources of discrimination.

Conclusion

Thus, HIV infection is in decreasing order though is one of the major infectious diseases in Nepal, and being chronic lifelong in nature, its impact is huge compared to other infectious diseases. People with high risk behavior and the spouse of the infected and affected couple need to be educated for primary and secondary preventive measures of the HIV infection. PLHIV should be educated that the timely initiation and continuous intake of antiretroviral therapy will not only prolong their survival but will also decrease the viral load and transmission of the disease. Provision of free antiretroviral treatment by the government of

Nepal is a step in the right direction, and it should be extended to the entire country, as antiretroviral treatment does change the quality of life of the positives as well as his/her family and the positive is able to get back to work and restart his/her livelihood.

Hence, ART service was found to be effective enough to increase the CD4 count significantly after 6 months of therapy.

It can be concluded that ART is effective enough in slowing the progression of HIV infection to AIDS and increasing the survival rate of positives with good performance. This study reflects the real situations of ART service in resource limited setting and help to promote the ART service to other parts of the country. On the basis of this study it can be recommended that HIV should be diagnosed earlier so that ART can be started in appropriate time.

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References

CDCP 1992. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep **41:** 1-19.

CDCP 2002. Guidelines for using Antiretroviral Agents among HIV-infected Adults and Adolescents. Morbid Mortak Weekly Rep **51:** 7.

Dhakal K.S. and Aryal B. 2012. Assessment of impact of Antiretroviral Therapy on quality of life among people living with HIV and AIDS. International Journal of Pharmaceutical and Biological Archives. **3**(3):534-538

Gautam H., Bhalla P., Saini S. and Dewan R. 2007. Correlation between Baseline CD4+ T-lymphocyte count and plasma viral load in AIDS positives and their early clinical and

immunological response to HAART: A Preliminary Study. Journal of Acquired Immune Deficiency Syndrome **45:** 183-192.

Kumarasamy N., Vallabhaneni S., Cecelia A. J., Yepthomi T., Balakrishnan P. and Saghayam S., et al. 2006. Reasons for modification of generic highly active antiretroviral therapeutic regimens among positives in Southern India. Journal of Acquired Immune Deficiency Syndrome **41:** 53-8.

Moore R. D. and Keruly J. C. 2007. CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virologic suppression. Clin Infect Dis **44:** 441-446.

NCASC 2012. Factsheets on HIV and AIDS, National Center for AIDS and STD Control, Ministry of health, Govt of Nepal, 2012.

Paudel B.N., Chaudhary S.R., Sharma S., Dhungana G.P. and Paudel P. 2009. Antiretroviral Service to HIV positives of low CD4 count in Seti Zonal Hospital. Journal of the Nepal Medical Association **48**: 173 pg 24-7.

Pirzada Y., Khuder S. and Donabedian H. 2006. Predicting AIDS-related events using CD4 percentage or CD4 absolute counts. AIDS Res Ther **3:** 20.

Sharma S., Dhungana G.P., Pokherel B.M. and Rijal B.P. 2009. Clinical features of HIV/AIDS and various opportunistic infections in relation to antiretroviral status among HIV seropositive individuals from Central Nepal. Kathmandu University Medical Journal, Vol. **7**, No. 4, Issue 28, 355-359.

Sunita K., Ram Babu Ch., Baratha J. N., Suresh B. K., Harika C. V., and Phani R. S. Ch. 2011. Effect of ART in attendees of Voluntary counseling and testing center of govt. general hospital, Guntar, Andra Pradesh, India. International Journal of Science Innovations and Discoveries, **1**(3): 451-462.

Tiwari B. R., Ghimire P., and Malla S. 2008. Study on CD4 cell responses in HIV infected subjects in Nepal. Nepal Medical College Journal **10**(1): 45-47.

Valdiserri R.O., Moore M., Gerber A.R., et al. 1993 A study of clients returning for counseling after HIV testing implications for improving rates of return. Public Health Report. **108:** 12-8

WHO, UNAIDS, AIDS Epidemic update 2011.

Woldemedhin B., and Wabe N.T. 2012. The Reason for Regimen Change Among HIV/AIDS Positives Initiated on First Line Highly Active Antiretroviral Therapy in Southern Ethiopia. North American Journal of Medical Science **4**(1): 19–23.

Wright S.T., Carr A., Woolley I., Giles M., Hoy J., Cooper D.A. and Law M.G. 2011. CD4 cell responses to combination antiretroviral therapy in positives starting therapy at high CD4 cell counts. **58**(1):72-9.

Infectivity of *Theileria annulata* in *Hyalomma* ticks of Eastern Terai districts, Nepal

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Abstract

The study was conducted to assess the prevalence of *Theileria* infection in *Hyalomma* tick vectors collected from cattle host to record the natural infection level of theilerial parasite in the field condition. A total of 174 male and 298 female of *Hyalomma marginatum issaci* ticks were collected from three districts Sunsari, Morang and Jhapa of Eastern Terai of Nepal during 2004-2005. These *Hyalomma* ticks were dissected and their salivary glands were stained with Methyl Green Pyronin (MGP) stain. 472 *Hyalomma* ticks were screened and 114 (24.15%) were found positive for *Theileria*. The percent of *Theileria* positive ticks were 8.62, 27.35 and 20.63 from Sunsari, Morang and Jhapa respectively. The prevalence, abundance and intensity of *Theileria annulata* infection were higher in female *Hyalomma* ticks (27.18%, 6.61 and 24.31 respectively) than male *Hyalomma* ticks (18.97%, 3.60 and 19.00 respectively).

Keywords*: Cattle parasite, Intensity, Methyl Green Pyronin (MGP) stain, Salivary gland*

Introduction

Bovine tropical theileriosis caused by *Theileria annulata* and transmitted by *Hyalomma* sp. is an economically important disease of cross-bred cattle which causes heavy economic loss in terms of high morbidity and mortality, loss of productivity and indirect losses due to cost of control measures. This posed a major challenge in the dairy pockets of Eastern Terai Region of Nepal where both the vectors as well as the parasites have been identified (Acharya and Pradhan 1996, Shrestha and Singh 2000 and RVL 2001/02). Occurrence of theileriosis shows

seasonality and the disease is mostly observed in summer from March to October. This is related to the activity of ticks' population which varies with climatic conditions viz. temperature, rainfall and humidity. Epidemiological studies in India have shown that young calves are highly susceptible to the disease. Depending upon the quantum of infection, these calves either suffer severely and die of the disease or react and recover. Breakdown of the preimmune status following stress of pregnancy, parturition, lactation, inter-current disease conditions and environmental factors is reportedly responsible for the occurrence of clinical form of the disease amongst adult exotic and crossbred cattle. Over 250 million cattle, in endemic areas were estimated to be at risk from *T.annulata* infection (Robinson 1982). Because of different cattle breeds, different tick vectors with different biology from one region to another and regional and seasonal variations, the epidemiology of tropical theileriosis is not the same everywhere. The detection of *Theileria* infection rate and intensity of infection in the vector ticks is an important component in the study of epidemiology of theileriosis. Work on this aspect was initiated with *T. parva* in Kenya and with *T.annulata* in Sudan (Walker et al. 1983) and India (Sangwan et al. 1986).

Materials and Methods

This study was conducted in Sunsari, Morang and Jhapa districts of Eastern Terai of Nepal during May 2004 to June 2005. *Hyalomma* ticks were collected manually from different part of cattle body. These ticks were preserved in 70% alcohol containing 5% glycerin. The preserved ticks were examined for their morphological characteristics under stereoscopic microscope and identified according to the figures and key described by Acarology Division, IMR 1995, Kaiser and Hoogstraal 1964, Morel 1989 and MAFF 1986. The *Hyalomma* ticks were dissected under a stereoscopic microscope according to the procedure of Blewett and Branagan (1973). Salivary glands were stained by Methyl Green Pyronin (MGP) staining method described by Irvin et al. 1981. Fixed in Cornoy's fixative, cleared in Xylene and mounting in DPX.

For assessment of infectivity rates of *Hyalomma* ticks, number of infected acini per tick were counted and recorded for both male and female ticks on the basis of the characteristics of the infected acini described by Walker et al. (1979). The degree of infection with *Theileria annulata* were expressed as prevalence [(number of infected ticks/number of ticks examined)

x100], abundance (number of infected acini/number of ticks examined) and intensity (number of infected acini/number of ticks infected) as per Margolis et al. (1982).

Statistical Analysis

The infectivity of bovine tropical theileriosis was analysed using chi-square (χ^2) test by F. Yates correction method.

Results

Out of a total of 472 *Hyalomma* ticks screened for the presence of *Theileria*, 114 ticks were positive for *Theileria.* Thus the general prevalence was 24.15% of the total ticks screened in the Eastern Terai of Nepal. The theilerial mass/sporoblast in the infected cell cytoplasm took greenish blue colour (Plate 1). The increase in size of the infected acinus showed great variations. In *Theileria* positive hypertrophied acini, the normal acinar cell nuclei were pushed towards one side. Hypertrophied nucleus of the infected cell was either in the center of the *Theileria* mass or at the periphery (Plate 1).

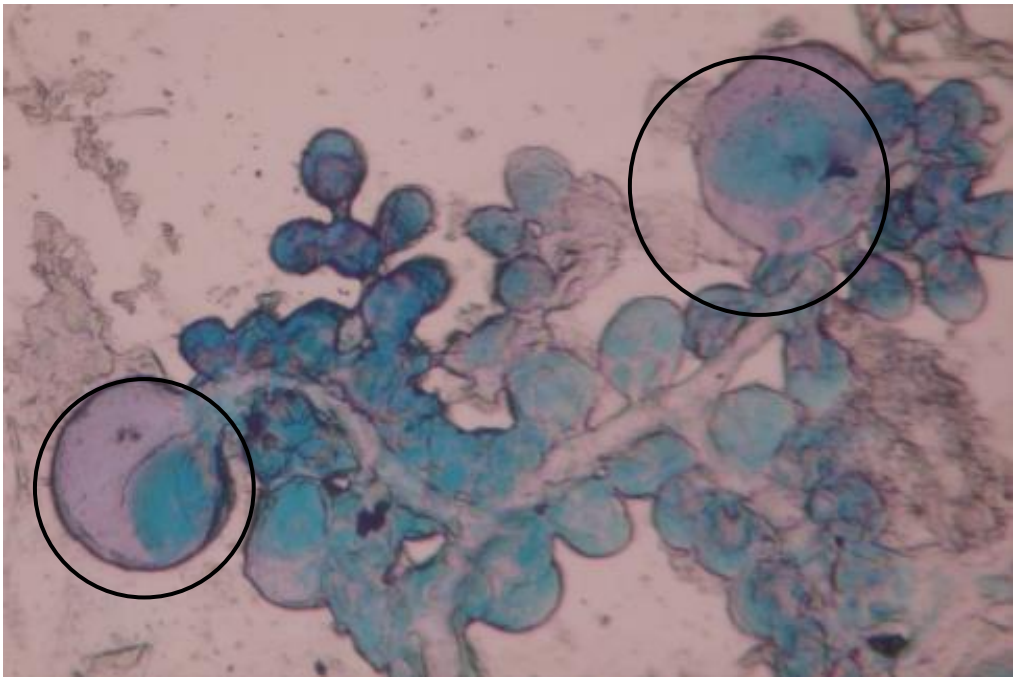

Plate 1. MGP stained salivary gland showing *Theileria* sporoblast infected acini harvested from *Hyalomma* tick (1000x)

In Eastern Terai Region a total of 58 *Hyalomma* ticks were examined from Sunsari district and 5 (8.62%) of them were *Theileria* positive. From Morang district, out of 351 *Hyalomma* ticks examined 96 (27.35%) were found positive. Of a total of 63 *Hyalomma* ticks examined from Jhapa district, 13 (20.63%) showed *Theileria* positivity (Figure 1).

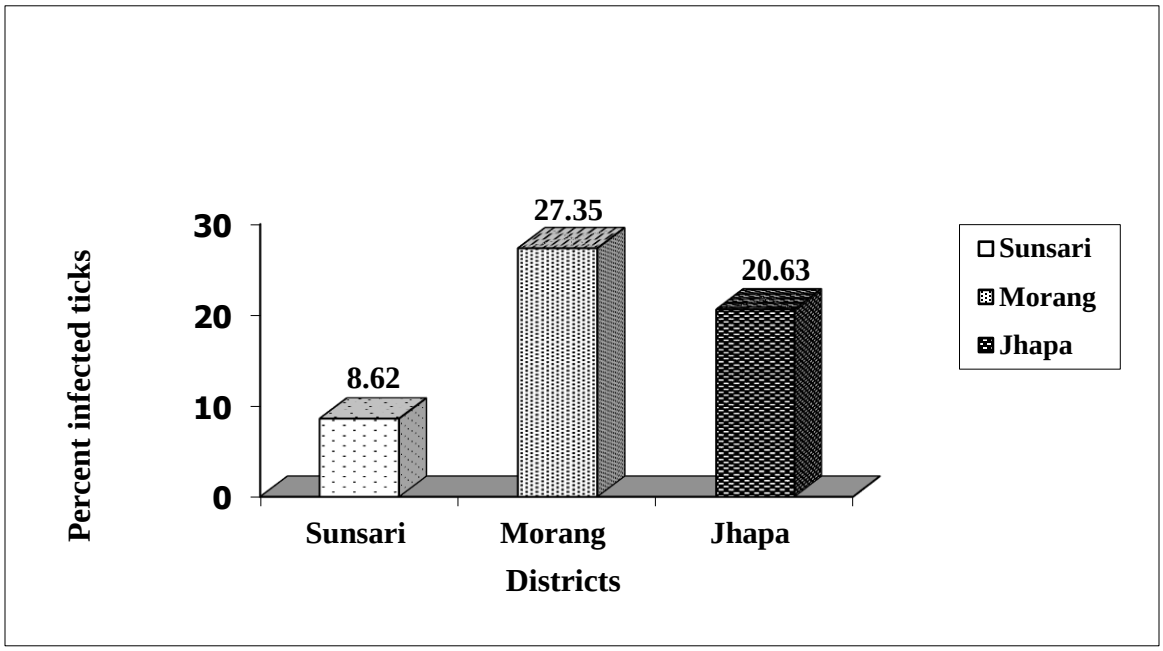

Figure 1. *Theileria* infection rate in *Hyalomma* ticks of Eastern Terai of Nepal

The prevalence, abundance and intensity of *Theileria* infection were found to be higher in female ticks (27.18%, 6.61 and 24.31 respectively) than male ticks (18.97%, 3.60 and 19 respectively) (Table 1).

Table 1. Prevalence of *Theileria annulata* infection in *Hyalomma marginatum issaci* of Eastern Terai of Nepal

Category	No. of ticks examined	No. of infected ticks	Total infected acini	Degree of salivary gland infection		
Sex of ticks				Prevalence[a] %	Abundance[b]	Intensity[c]
Male	174	33	627	18.97	3.60	19.00
Female	298	81	1969	27.18	6.61	24.31
χ^2 Value	-	4.048	-	-	-	-

[a]Total no. of infected ticks/total no. of ticks examined ×100, [b]Total no. of infected acini/total no. of ticks examined, [c]Total no. of infected acini/total no. of infected ticks.

Discussion

The present study indicates that theileriosis is now endemic in Morang district. Similar findings were reported by the studies made in the context of Eastern Terai of Nepal in the past (Acharya and Pradhan 1996, Shrestha and Singh 2000). Prevalence of the theileriosis is assumed to be much higher than that reported on the basis of clinical cases. Animals were treated on the basis of clinical symptoms alone in the field condition and many cases remain undiagnosed. Cases of theileriosis occurred between the months of March and October. It is a vector borne disease and a peak of tick activities during hot and humid climate of summer and rainy seasons appears to be responsible for this seasonal increase in clinical form of theileriosis. Observations made by Flatch and Ouhelli (1992) were similar and Radostits et al. (1994) also pointed out the importance of air temperature in variation of tick population and thus the seasonal incidence of the disease. Occurrence of 24.15% of the theileriosis as found in the present study is consistent with the epidemiological findings from RVL 2001/2002, and from India which had shown the high susceptibility in small age group (Beniwal et al. 1997). *Hyalomma marginatum issaci* identified in this study is the specific vector of bovine tropical theileriosis. With the change in ecological, agronomical and managemental practices, the microclimate and ecological niche of livestock ticks are undergoing changes leading to disturbances in tick populations. In this study, almost in all animals, the number of tick infestation per animal was more than fifty. *Hyalomma marginatum issaci*, the vector tick for theileriosis, was identified from cattle of three districts Sunsari, Morang and Jhapa of Eastern Terai, Nepal. In the presence of parasites as well as the vectors, it can be said that Eastern Terai region is endemic for theileriosis. However, very scanty work has been done regarding quantitative epidemiology of bovine tropical theileriosis in Nepal. Detection of theilerial sporoblasts in the salivary glands of vector ticks are the most useful tools for the epidemiological studies of theileriosis. The tick collection methods employed in the present study were found adequate and feasible for the quantitative assessment of *Theileria* infection in the field. It is very difficult to guess the physiological age of an adult male tick as its size does not vary appreciably with the age and unlike female ticks it can feed repeatedly on the host for a long period. Due to this factor, a direct collection of engorging ticks from the host for theilerial assessment is not preferred. However, several workers (Bouattour et al. 1996, Kumar 2000) have dissected engorging ticks collected from

the hosts as it is far more convenient and still accurate for the female ticks. Whole salivary gland staining with MGP worked satisfactory for assessing *Theileria* infectivity in ticks. The most consistent diagnostic characteristics of the presence of *Theileria* masses i.e. sporoblasts in the salivary gland acinus as indicated by the hypertrophy of the acinus and of infected acinar cell nucleus along with the presence of diffuse theilerial mass in the cytoplasm observed in this study, confirmed the earlier observations of this feature (Blewett and Branagan 1973, Walker et al. 1979, Sangwan et al. 1986). RVL (1996) was also recorded 21(43.75%) positive cases of *T. annulata* of the total 48 cases. In the present study, 114 (24.15%) out of 472 *Theileria* positive cases was recorded. The prevalence percent, abundance and intensity of theilerial infection in *Hyalomma* ticks were higher in female ticks than male ticks. This finding will contribute to a better knowledge of the epidemiology of *T. annulata* infection in Eastern Terai of Nepal.

Conclusion

Bovine tropical theileriosis has remained a major health hazard for the exotic and crossbred cattle since the introduction of large scale crossbreeding of native cattle in Nepal with the dairy breeds. In addition to the great economic losses, the disease is also inhibiting the potential for milk production by making farmers reluctant to opt for cross breeding of their cattle. Although bovine tropical theileriosis is widespread in Eastern Terai of Nepal and is a serious challenge to a livestock development programmes, its epidemiology has not been adequately studied. Study of prevalence of *Theileria* both in cattle and in the vector ticks is an important component to know the epidemiology of theileriosis. The epidemiology of the disease has not been studied so far in the Eastern Terai of Nepal where the vector ticks are found in abundance. Out of a total of 472 ticks screened for the presence of *Theileria*, 114 (24.15%) ticks were positive for *Theileria*. In order to know the level of *Theileria* infection in *Hyalomma* ticks in the Eastern Terai of Nepal, frequency distribution of *Theileria* positive acini per positive tick was worked out. 85.09 percent ticks showed one to five positive acini and 14.91 percent ticks showed more than five positive acini.

Recommendations

Based on the research findings, following recommendations could be made:

* Infectivity of *T. annulata* in *Hyalomma* ticks was high in the Eastern Terai of Nepal. The occurrence of clinical cases of theileriosis in cattle of the Eastern Terai of Nepal may be more. So, further investigation should be carried out.
* Infectivity of *T. annulata* was higher in female ticks than male ticks in all three districts of the Eastern Terai of Nepal. This study should be continued.
* The sero-prevalence study of theileriosis in cattle should be carried out.
* Introduction of susceptible crossbred cattle in Eastern Terai of Nepal needs rethinking.
* This study should be carried out again to find the effect of *T. annulata* in economy status of Nepal.

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References

Acharya, K.P. and Pradhan, S.M.S. 1996. Study of blood protozoan diseases with special reference to theileriosis in Eastern Terai of Nepal. Bull. Vet. Sc. & A.H. Nepal **24:** 55-58.

Beniwal, R.K., Nichani, A.K., Sharma, R.D., Rakha, N.K., Suri, D. and Sarup, S. 1997. Responses in animals vaccinated with *Theileria annulata* (Hisar) cell culture vaccine Trop. Anim. Hlth. Prod. **29:** 109S-113S.

Blewett, D.A. and Branagan, O. 1973. The demonstration of *Theileria parva* infection in intact *Rhipicephalus appendiculatus* salivary glands. Trop. Anim. Hlth. Prod. **5:** 27-34.

Bouattour, A., Darghouth, M.A. and Benmiled, L. 1996. Cattle infestation by *Hyalomma* ticks and prevalence of *Theileria* in *H.detritum* species in Tunisia. Vet Parasitol. **65:** 233-245.

Flach, E.J. and Ouhelli, H. 1992. The epidemiology of tropical theileriosis (*Theileria annulata* infection in cattle) in an endemic area of Morocco. Vet. Parasitol. **44:** 51-65.

IMR. 1995. Institute of medical Research, Acarology Division. Key to the families and genera of the Southeast Asian Metastigmata (ticks). Kualalumpur, Malaysia.

Irvin, A.D., Boarer, C.D.H., Dobbelaere, D.A.E., Mohan, S.M., Marake, R. and Ocama, J.G.R. 1981. Monitoring *Theileria parva* infection in adult *Rhipicephalus appendiculatus* ticks. Parasitology, **82:** 137-147.

Kaiser, M.N. and Hoogstraal, H. 1964. The *Hyalomma* ticks (Ixodoidea, Ixodidae) of Pakistan, India and Ceylon, with keys to subgenera and species. Acarologia, **6:** 257-286.

Kumar, M. 2000. Studies on some epidemiological aspects of bovine tropical theileriosis in Haryana, M.V.Sc. Thesis. CCS Haryana Agric. Univ. Hisar, India.

MAFF. 1986. Ministry of Agriculture, Fisheries and food. Manual of veterinary parasitology Laboratory Techniques. Reference book 418, HMSO, London.

Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M. and Schad, G.A. 1982. The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists) J. Parasitol. **68**:131-133

Morel, P. 1989. Tick borne diseases of livestock in Africa. In: Manual of Tropical veterinary parasitology (translated by Fisher, M.S. and Say, R.R.), CAB International Wallingford, UK. p. 299-329.

Radostits, O.M., Blood, D.C. and Gay, C.C. 1994. Veterinary medicine. 8th ed. ELBS and Baillere Tindall, London, p. 1171-1179 and 1207-1212.

Robinson, P.M. 1982. *Theileria annulata* and its transmission- A review. Trop. Anim. Hit. Prod. **14:** 3-12.

RVDL (Regional Veterinary Diagnostic Laboratory), Annual Report. 1996, 1995/96, RVDL, Biratnagar, p. 56.

RVL 2001/2002, Annual technical Report. Regional Veterinary Laboratory, Biratnagar.

Sangwan, A.K., Chhabra, M.B. and Samantaray, S. 1986. *Theileria* infectivity of *Hyalomma* ticks in Haryana, India. Trop. Anim. Hlth. Prod. **18:** 149-154.

Shrestha, S.P. and Singh, U.M. 2000. Identification of ticks in relation to blood protozoan diseases in cattle. In: Neupane, S.R.P., Rai, A.K. and Bhurtel, R. eds. Proceedings of the fourth National Workshop on Livestock and Fisheries Research in Nepal, NARC, Khumaltar, p. 165-167.

Walker, A.R. and Mckeller, S.B. 1983. The maturation of *Theileria annulata* in *Hyalomma a. anatolicum* stimulated by incubation or feeding to produce sporozoites. Vet. Parasitol **13:** 13-31.

Walker, A.R., Latif, A.A., Morzaria, S.P. and Jongejan, F. 1983. Natural infection rates of *Hyalomma anatolicum anatolicum* with *Theileria* in Sudan Res.Vet. Sci. **35:** 87-90.

Walker, A.R., McKeller, S.B., Bell, L.J. and Brown, C.G.D. 1979. Rapid quantitative assessment of *Theileria* infection in ticks.Trop. Anim .Hlth. Prod. **11:** 21-26.

Growth response of rainbow trout (*Oncorhynchus mykiss*) on substitution of shrimp meal by different protein sources

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Abstract

Shrimp meal (SM) is costly major protein source of trout feed in Nepal. The present work is a feeding trial to develop cost effective formulated trout feed replacing SM by synthetic amino acids (SA), silkworm pupae (SWP) or silkworm moth (SWM). The four diets were prepared iso-nitrogenous with 44.44 ($\pm$0.5) % protein. The trial was conducted for eight weeks in three replications for each diet in random block design (RBD). For which, fry (average 0.31g) were stocked in twelve cages (0.5m^3) kept in a raceway pond at a density of 2000 fry m^{-2}. The feed was given 8 to 10 times a day to satiation. SWP feed showed significantly higher weight gain ($p<0.5$). Mortality rates of fry were found statistically insignificant ($p>0.05$) in SM, SWP and SWM feed but significant highest mortality rate (77%) was observed in synthetic amino acid. Lowest feed conversion ratio (FCR) was 1.33 recorded in SWP feed. However, FCR was calculated statistically insignificant ($p>0.05$) in all four diets. Study concluded silkworm meal could be a proper substitute in rainbow trout feed if it is locally available.

***Keywords**: FCR, protein, silkworm meal, survival rate, weight gain*

Introduction

Nepal has diverse native cold water fishes but commercial production of them has not yet been started due to the lack of overall technical knowhow. Exotic rainbow trout (*Oncorhynchus mykiss*) is introduced (1990) and commercially exploited (from 1995 and 1998) in Nepal to expedite comparative advantage of climatic condition and coldwater resource. Trout farming is an intensive system with the use of 'nutritionally-complete formulated diets'. Aquaculture system becomes economically profitable with the input of low cost nutritionally balanced feed. As fish feed occupies about 40-60% of total cost of fish culture (Akiyama et al. 1992). Formulation of fish feed from locally available inexpensive ingredient is immensely required for the expansion of trout industry in Nepal. Use of SWP in the substitute of Fish Meal (FM) was reported in several research works. Rangacharyulu *et*

al. (2003) and Hossain et al. (1997) used fermented SWP, fish silage and boiled silkworm pupae in place of fishmeal. Nanda (1967) also reported processed silkworm pupae as excellent source of protein in fish feed. Nataraj and Basavanna (1996) reported protein concentration of silkworm pupae as supplement/substitute to fish meal. Singh et al. 2008 reported that dead silkworm pupae and moths could also be used as fish feed. Swamy and Devraj (1994) reported higher fat and protein content in silkworm pupae than plant proteins.

Materials and Methods

Experimental site and diets

The experiment was conducted for 8 weeks at private farm in Dhading. The fry were graded for uniform size and length (average 0.31 g and 2.9 cm). Four feed like shrimp meal (SM), synthetic amino acids (SA), silkworm pupae (SWP) and silkworm moth (SWM) were prepared iso-nitrogenous with 44.44% ($\pm$0.5) protein. 12 cages (0.5 m^3) were installed in a raceway pond (8 m x 1.25 m x 0.9 m) in three replications for each diet in RBD. Five hundred fry were stocked in each cage with the regular inflow of water. The feed was given 8 to 10 times a day to satiation. Prior to study work, fry were kept starved for 24 hour and feed was given at a required percentage of body weight. After two week interval, fifty fry from each cage were randomly collected for weight and length measurement. One way Analysis of Variance, ANOVA was applied using Tukey Multiple range test in SPSS software to evaluate growth response on different protein source feed at 95 % confident level.

Table 1. Composition and proximate analysis of experimental diets (g/100g diet).

Ingredients	Diet-1	Diet-2	Diet- 3	Diet- 4
	SM[a]	SAA[b]	SPM[c]	SMM[d]
Big Shrimp	50	-	-	-
Lysine+Meth	-	30+10	-	--
Silkworm pupae	-	-	50	-
Silkworm moth	-	-	-	50
Soybean powder	35	35	35	35
Wheat powder	15	25	15	15
Vitamin pemix	1	1	1	1
Mineral premix	1	1	1	1
Vitamin-c	0.1	0.1	0.1	0.1
Proximate composition				
Moisture	8.97	11.54	10.35	3.66
Ash	11.67	5.06	5.65	7.13
Crude fats	8.62	6.48	17.01	20.85
Crude protein	38.27	48.47	42.68	46.01
Crude fiber	2.65	1.86	2.8	3.11

[a]SM = shrimp meal, [b]SAA = synthetic amino acids, [c]SPM = silkworm pupae, [d]SMM = silkworm moth.

Source: Fisheries Research Division, Godawari

Results and Discussion

The final weight of fry was found significantly different fed with four different diets. Growth was highest with 2.34 g mean weight per fry in silkworm pupae feed. Highest specific growth rate (SGR) was recorded in silkworm pupae (1.26) followed by shrimp meal (1.11) and silkworm moth (0.99). But the lowest specific growth rate was recorded in synthetic amino acid (0.41). SGR was recorded significantly different in all the test protein diets ($p<0.05$) (Table 2).

Table 2. Mean initial body weight, weight gain, FCR, SGR and survival of *O. mykiss* fry fed test diets containing different protein sources for 8 weeks.

	Diet			
	SM	SAA	SPM	SMM
Mean Initial Weight (g/fish)	0.32[a]	0.28[a]	0.29[a]	0.34[a]
Mean Final Weight (g/fish)	2.02[a]	0.55[b]	2.34[c]	1.76[d]
SGR	1.11[a]	0.41[b]	1.26[c]	0.99[d]
FCR	1.46[a]	3.75[a]	1.33[a]	2.46[a]
Survival (%)	96[a]	23[b]	85[a]	84[a]

*Values in a same row having the different superscript are significantly different ($p < 0.05$: Tukey multiple range test).

Mean FCR was recorded 1.46, 3.75, 1.33 and 2.46 for shrimp meal, synthetic amino acid, silkworm pupae and silkworm moth respectively (Table 2). The lowest FCR value was found for silk worm pupae. Rangacharyullu et al. (2003) also reported lower FCR (2.1) and higher SGR (2.39) in silk worm pupae compared to fish meal with FCR (3.16) and SGR (2.2). Silkworm meal showed also good growth and feed conversion in carps (Jeyachandran and Raj 1976; Erencin, 1976; Borthakur et al. 1998; Nandeesha et al. 1990). The highest survival (96%) was found in shrimp meal and lowest (23%) in synthetic amino acids (Table 2). Survival rate was found lower in silkworm pupae meal (85%) than that of shrimp meal (96%) in present study. This might be due to some experimental error, as Rangacharyullu *et al.* (2003) reported better survival rate in silkworm pupae (84.2%) compared to fish meal (67.5%). High mortality and poor growth was recorded in SAA (diet 2). Reduced growth and high mortality was reported at 30% lysine and 10% methionine (Yvonne et al. 1957). According to Yvonne et al. excess lysine was responsible for lysine toxicity with poor growth and feed utilization. The normal quantity of methionine should be around 1.0-2.35% in rainbow trout (Walton et al. 1982).

Conclusion

FCR and SGR were found highest in silkworm pupae among four diets. The synthetic feed like methionine and lysine supplemented with plant protein (diet 2) revealed poor growth and survival rate. Hence, silkworm pupae meal can be used as alternate to replace shrimp meal in trout feed.

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References

Akiyama, D.M., Dominy, W.G. and Lawrence, A.L. 1992. Penaeid shrimp nutrition. In: Fast, A.W., Lester, L.J. (eds.), *Marine Shrimp Culture: Principles and Practices. Elsevier,* Amsterdam: 535-568.

Borthakur, S. and Sarma, K. 1998. Protein and fat digestibility of some non-conventional fish meal replacers in diets of fish *Clarius batrachus* (Linnaeus, 1758) Environment and Ecology, **16**(2): 368-371.

Erencin, C. 1976. Studies on feeding and performance of carp varieties and their crosses in Cifteler, Turkey. Verlag Paul Parey

Hossain, M.A., Nahar, N. and Kamal, M. 1997. Nutrient digestibility coefficients of some plant and animal proteins for rohu (Labeo rohita). Aquaculture **151**: 37–45.

Jeyachandran, P. and Raj, S.P. 1976. Experiments with artificial feeds on *Cyprinus carpio* fingerlings. Indian Journal of Inland Fishery **8**: 33-37.

Nanda, B. 1967. Some newer sources of ingredients for Poultry feed. Poultry Guide **4**: 42-43.

Nataraja,G. and Basavanna,H.M., 1996. Proteins from silkwormpupae.silkworm. Information Bulletin, **1** (1): 29-30.

Nandeesha, M.C., Srikantha, G.K., Keshavanatha, P., Varghesea, T.J., Basavarajaa, N. and Dasa, S.K. 1990. Effect of non-defatted silkworm pupae in diets on the growth of Common Carp (*Cyprinus Carpio).* Biological Wastes **33**(1): 17-23.

Rangacharyulu, P.V., Paul, B.N., Yashoda K.P., Rao, R.J., Mahendrakar, N.S., Mahanty, S.N. and Mukhopashyay, P.K. 2003. Bioresource Technology **86**(1): 29-32.

Singh, D.M., S. Dahal, P.B., Shrestha, C.R. and Mishra, M. 2008. Extension strategies for Rainbow Trout (*Oncorhynchus mykiss*) aquaculture development in Nepal. In: T.B. Gurung (ed.) Proceedings of the workshop on "Rainbow trout farming scaling-up strategies in Nepal", 136-139.

Swamy, H.V.V. and Devaraj, K.V. 1994. Nutrient utilization by common carp (***Cyprinus carpio*** Linn) fed protein from leaf meal and silkworm pupae meal based diets. Indian Journal of Animal Nutrition, **11** (2): 67-71

Walton, M.J., Cowey, C.B and Adron, J.W. 1982. Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. Journal of Nutrition 112: 1525-1555.

Yvonne N. Chiu, Y.N., Austic, R.E. and Rumsey, G.L. 1957. Interaction among dietary minerals, arginine and lysine in rainbow trout (*Oncorhynchus mykiss*). Fish physiology and Biochemistry **4**(1): 45-55.

Human-monkey interface in Arkhale and Nayagaun, Gulmi, West Nepal

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Abstract

The ecology of existing monkey species in Arkhale and Nayagaun Village Development Committee of Gulmi district Nepal was studied with human versus monkey interface. Two species of monkeys, Rhesus monkey (*Macaca mulatta*) and Hanuman Langur (*Semnopithecus entellus*) were found in study area. Monkey population was counted by direct observation. Four troops of rhesus monkey with a population of 128 and a troop of Hanuman Langur with 14 individuals were recorded. Rhesus monkey was considered most crops damaging (65% respondents) while physical hurt and harassment (27%) were also perceived monkey related problem in the study area. Shouting and chasing (30%) with using stone and catapult (24%) were the common local deterrent method against monkeys. Monkey problem was increasing (82% of respondents) in the area. Worth compensation (39%) would be the effective measure to reduce human-monkey conflict in the studied area. Both primary and secondary data were used for analysis. Quadrates were laid to explore the habitat and vegetation pattern of natural forest.

Key Words: *Rhesus, Hanuman langur, conflict, remedial measures, Nepal*

Introduction

Monkeys are included under the sub-order Simiae of order primates. Monkeys according to the geographical distribution are further categorized into two types: New World monkeys and Old World monkeys. The New World monkeys lack cheeks pouches and nostrils are open to side rather than down. Area between the nostrils is wide and flat. Some of them have prehensile tail and non-have callous pads on the buttocks, E.g. Spider monkeys, Capuchins,

etc. The Old World monkeys have protruded muzzle and well developed cheek pouches, nostrils set close together facing forward and downward. The tail is never prehensile and some species are tailless. Both the hands and feet are adopted for grasping (Chalise 1999).

In Nepal, only three species of monkeys (Hanuman Langur, Rhesus and Assamese Monkeys) are recorded (Chalise et al. 2005). The Rhesus monkeys (*Macaca mulatta* Zimmermann, 1780) are found freely ranging in wild as well as in urban religious places. The Langur monkeys (*Semnopithecus entellus* Dufresne 1797) are found freely ranging in wild forest and marginal areas of Nepal (Southwick et al. 1982). The other species Assamese monkey (*Macaca assamensis* McClelland 1840) reported from mid-hills and high Montana forest of Nepal, whose ecological and behavioral details are still largely unknown (Chalise 2006).

Human beings and wild monkeys share the common resources to meet daily needs in most part of the country. Human being often ignore them and exploit their habitat. It results the beginning of the monkey and local people conflicts. Local people often reports that monkey population is increasing due to habitat protection and wild lifers claimed that habitat has been continuously decreasing. Moreover, rapid increases in human population demand more space and resources resulting in the encroachment of ancestral habitat of wild fauna while expansion of agricultural area created a serious environmental problem. The scarcity of prefer food in natural habitat and habitat destruction compels monkeys to enter human interest area causing damage of food and clothes while food stealing and human harassment are common (Chalise 1999a).

Materials and Methods

Study Area

The study sites Arkhale and Nayagaun lie in south-western part of the Gulmi District of Lumbini Zone in the Western development region of Nepal. The district occupy the mid-hill region and located between latitude 22º55' N to 28º 27'N and longitude 83º10' E to 83º35' with the elevation between 465 m to 2690 m asl. These areas consist of forest predominantly subtropical and temperate characters. At higher altitude as well as south facing areas consists of pine forest patches while lower basins as well as north facing slopes consist of mixed forest of *Schima-Catanopsis* species. In general, main floral species observed in Arkhale and

Nayagaun consist of Pine (*Pinus ruxbergii*), Sal (*Shorea robusta*), Chilaune (*Schima wallichi*), Katus (*Catanopsis indica*), Uttis (*Alnus nepalensis)*, etc. Major wild animals reported are Leopard (*Panthera pardus*), Jungle cat (*Felis chaus*), Common langur (*Semnopithecus entellus*), Rhesus monkeys (*Macacca mulatta*), Common mongoose (*Herpestes edwardsii*), Porcupine (*Hystrix indica*) etc. The bird species are Owl (*Bubo bubo*), Black Kite (*Milvus migrans*), Vulture species, Eagle (*Spilornis cheela*), Kalij pheasant (*Lophura leucomelana*), Cuckoo (*Cuculus* sps.), Common myana (*Acridotheres tristis*), House crow (*Corvus splendens*), Woodpecker (*Picus* sps.), etc.

Sampling Method and Sample Size

Four wards were selected; two from each, Arkhale and Nayagaun VDC (wards 1 and 2 from Arkhale and 8 and 9 from Nayagaun). Fifty percent of the households from these wards were taken randomly for the survey.

Household questionnaires

A pre-tested and semi-structured questionnaire was used to collect the information from local villagers contained period of monkey visitation, monkey related problem, preventing methods used by the locals, possible remedial measures of conflict, flora and fauna of the area, etc.

Monkey's population and distribution study

Regular diurnal observation was done to locate the monkey species, numbers, age, sex and distribution in study sites. Observation was done without disturbing natural setting with the help of binoculars. Repeated observation was made to confirm the collected data on monkeys in their home range. Individuals were distinguished by their body colour, proportion and body size as described by Roonwal and Mohnot (1977).

Quadrate method

In the study area there was interruption of natural forest by human settlement, thus forest was fragmented into four patches. Each patches were divided into three transect of more or less equal difference and randomly Quadrate of 25×25m was laid to study the vegetation pattern of the natural forest. The local name of plant was identified by the experienced local persons and later scientifically enumerated in the Botany Department TU.

Data Analysis

Both descriptive statistics (percentages, frequencies) and inferential statistics were used to analyze the data. Household's questionnaires responses were carefully processed and arranged to make sense to researcher for scientific writing.

Results

General Distribution

A total of 4 troops of Rhesus monkeys were counted with 128 individuals and one troop of 14 individuals Langur were observed during the study period. The forest of Sitheni, Mulaghari, Khannichaur-Harrachaur, and GurungGaun each consists of one troop of Rhesus monkeys each (Table 1). Langur was found residing between the forest of Khannichaur-Harrachaur and GurungGaun (Table 2). No Langur troop was observed in the forest of Sitheni and Mulaghari.

Table 1. Population of Rhesus

Site	Number of troop	Total individuals	Ward/ Block
Sitheni	1	30	1/A
Mulaghari	1	23	2/B
Khannichaur	1	44	8/C
GurungGaun	1	31	9/D
Total	**4**	**128**	

Table 2. Population of Langur

Individual	Numbers
Adult male	1
Adult female	2
Sub adult male	3
Young adult female	2
Juvenile	4
Infant	2
Total	**14**

Troop Composition

Among the population of Rhesus monkey the first troop (A) in the forest of Sitheni composed of 30 individuals with 2 adult males, 6 adult females, 4 sub-adult males, 4 young-adult females, 9 juveniles and 5 infants. The second troop (B) in the forest of Mulaghari was composed of 23 individuals with 2 adult males, 4 adult females, 6 sub adult males, 4 Young-

adult females, 5 juveniles and 2 infants were found. The third troop (C) in the forest of Khannichaur-Harrachaur composed of 44 individuals with 3 adult males, 8 adult females, 7 sub adult males, 6 sub adult females, 14 juveniles and 6 infants. The fourth troop (D) which was found in the forest of GurungGaun composed of 31 individuals. Troop D consists of 3 adult males, 7 adult females, 5 sub-adult male, 3 young-adult females, 7 juveniles and 6 infants.

Among the total 128 individual of Rhesus, adult males were 10, adult females 25, sub-adult male 22, young-adult female 17, juveniles 35 and infants 19. Population composition of the monkey troops recorded in the study area showed the highest percentage of Juveniles i.e. 27% followed by adult female 20%, sub-adult male 17%, infants 15%, young-adult females 13%, and adult male 8% (Fig 1).

Population composition of langur troops recorded in the study area showed the highest percentage of Juveniles i.e. 29% followed by sub-adult male 22%. Percentage of young-adult male, infants and adult male was same i.e. 14%, followed by adult male 7% (Fig 2).

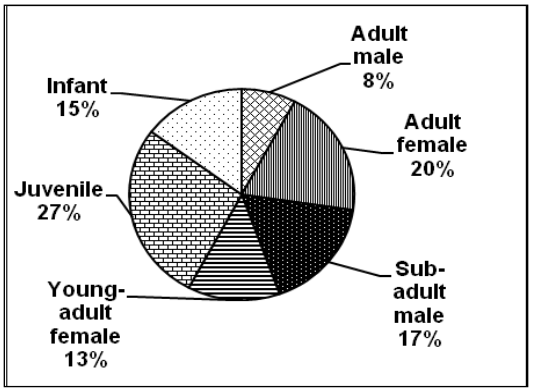


Figure 1. Group composition of Rhesus

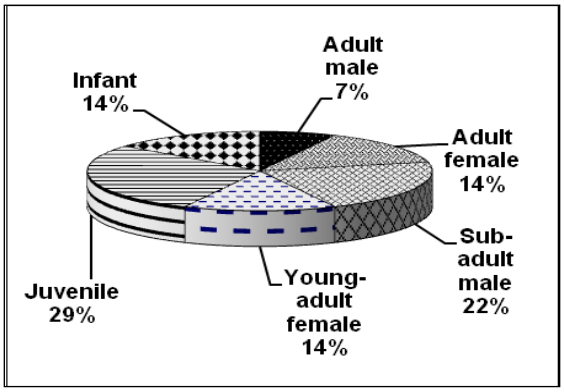


Figure 2. Group composition of Langur

Problem caused by monkey

Out of the total sample population (i.e. 242), one hundred and fifty five respondents (i.e. 64%) answered crop damage as the major problem. Physical hurt and harassment was the second with 65 respondents (i.e. 27%), whereas 22 respondents (i.e. 9%) were found with other problems due to monkey, like stealing household goods and some socio-economic burden. Some of the local people reported that they even had to quite their daily job to care

the crop field against monkeys, local children seems deprived of going to school as they have to guard their crop field all over the day during peak crop fruiting period.

Species of monkeys damaging more

Population of rhesus monkey was far higher (n=128) than that of the Langur (n=14) in the study area and the extent of damage was also high by the rhesus. Sixty five per cent of farmers in the study area reported that the rhesus monkeys were more damaging to crop and thirty five per cent of the respondents replied that both species of the monkeys equally damaged their crop (Fig 3).

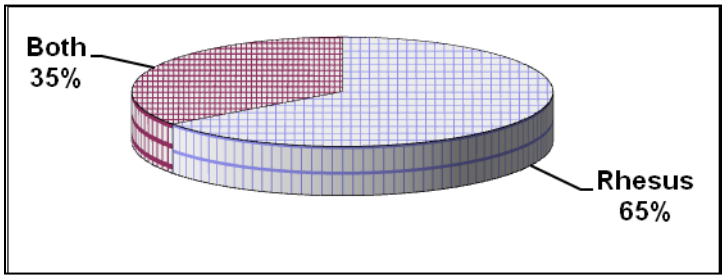


Figure 3. Crop damaging intensity of monkeys in Gulmi, Nepal

Percentage of Crop raiding by monkeys

From the analysis of crop depredation in the study area, it was found that maize was the highest (53%) attraction of monkeys that followed by wheat (23%), paddy (16%) and others (8%) like potato, fruits, mustard, millet, barley, pulses, etc. The extent of damage was also different in various stages of growth.

Cause of monkeys' problem

Thirty four, out of 242 respondents were of the opinion that monkeys were raiding to their crop field to feed because of less palatable food in the forest (Fig 4). People thought the increase of monkey population itself was the next major cause for the problem. Lack of arms and no provision of killing the monkeys, increase of planted forest area, crop field adjoining to forest etc. were other major issues marked as responsible to heighten the problem of monkey.

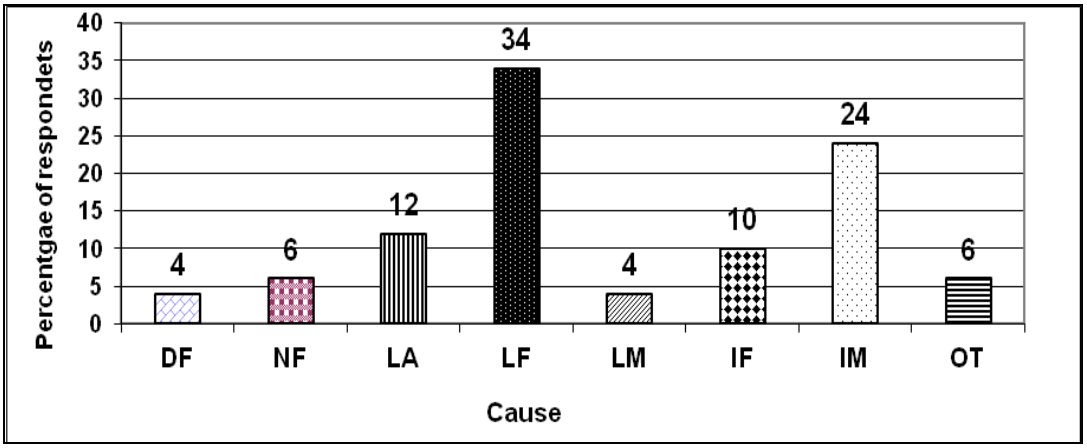
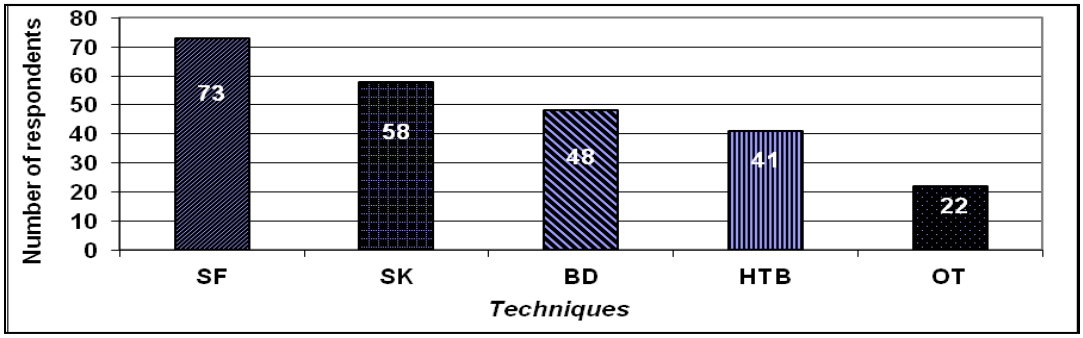


Figure 4. Local people perception towards increasing monkey problem

(Note: DF= Deforestation, NF=Crop fields near to forest, LA=Lack of arms, LF=Lack of food in forest, LM= Lack of manpower, IM=Increase of monkeys population, IF- Increase of forest, OT= Others)

Deterrent methods against Monkey

Local peoples adopted various methods to protect their crops form the monkeys. About 30% of the people shouted and followed monkeys to chase from the field and about 24% of the people used stone and catapult. Similarly, chasing the monkeys by dog as well as hitting tin boxes was other methods used by local people. Some of the people said that they used gun, fire cracker and catapult to chase the monkey. Local farmers guarded their crop fields all over the day in local huts called *Tharku*. Lighting fire in the edge of field, playing music through cassette player or radio with loud sound as well as planting thorny plants and non-palatable crops were the major strategies adopted by the farmers to keep away monkeys (Fig 5).



Note: SF=Shouting and following, SK= Stone and catapult, BD= By dog, HTB= Hitting tin boxes, OT=Others

Figure 5. Methods applied to chase out monkeys from the human crop fields.

Remedial Measures

Most of the people of Arkhale and Nayagaun (39%) suggested worth compensation as best remedial measure of monkey problem. Catching and killing (26%), mass chasing (21%) and alternative crop (14%) were the other measures suggested by local farmers.

Discussion

Among the three different species of monkeys reported from Nepal (Chalise 2006), only two species of Monkey Rhesus monkey (*Macaca mulatta*) and common Hanuman Langur (*Semnopithecus entellus*) were observed in Arkhale and Nayagaun of Gulmi. Assamese monkey (*Macaca assamensis*) was not found in the study Area. Rhesus monkey was in highest number (128) among the two species available locally. It may be due to its most commensal characters to human and crop raiding. Langur population was found to be 14. The lesser number of Hanuman Langur may be due to the small natural leafy habitat and being less commensally to human (Khatry 2006).

Crop raiding was found as a major cause of conflict caused by monkeys though physical hurt and harassment, grabbing and taking of food materials were also reported. Among the respondents crop raiding was reported by 64%. But the extent of crop raiding was found to be different in different areas. Crop raiding was found to be highest in the village near to the forest of GurungGaun (73%) followed by the village of Khannichaur-Harachaur. Village near to the forest of Mulaghari reported least to the crop raiding (59%). Higher extent of crop raiding in former two is due to the settlement and crop field very near to the forest. Khatry (2006) found that 76% of the respondents of Vijayapur area of Dharan reported the crop raiding as the major problem. Similarly, the study of McCourt (2005) showed that 92% respondents of Hetauda were found to be suffered from crop damage from monkeys. 87% of respondents complained the harassment by monkey by taking food spilling or eating from the kitchen, porch or roof.

Of the different crops raided by monkeys, maize was of highest preference (53%) of monkey. It was followed by wheat 23%, paddy 16% and others 8%. Regarding the monitory loss also maize occupied highest loss (48%); fruits shared 17%, paddy 12%, wheat 11%, potato 4%, millet 3%, mustard 3% and pulses 2%. Barley has least share in the monitory loss with about 0.5%. Raid percentages of crop also followed nearly similar trend. Maize was highly raided

crop (21.5%) of the total yield destructed by monkeys followed by wheat (20%), paddy (12.5%), fruits (10.22%), millet (9.33%), potato (9.2%), mustard (4.17%), barley (4%) and pulses (1.94%). This finding is also supported by the previous researches of Chalise (1997, 1999, 2001, and 2003). Chalise (2001) and Chalise and Johnson (2005) reported that crop depredation proportion by monkeys is different in different crops in different localities. Chalise (2010) reported that the crop raiding data can be collected in combine as the locals are unable to report categorically crop loss done by Langurs, rhesus and Assamese. The monkeys most favored crop is maize (43%) while potato (20%), millets (16%) and wheat (13%) for assamese monkeys in Langtang National Park.

As perceived by local farmers of Gulmi, lack of natural food in the forest was the major cause compelling monkey to raid the crop field. Thirty four, out of 242 respondents were of the opinion that monkeys came to their crop field to eat as there was no food in the forest. People thought the increase of monkey population was the next major cause for the increase of problem. Lack of different repellent tools and location of cropland were other major components responsible to heighten the problem of monkey in the area. Different preventive measures were applied to deter monkey. Local peoples usually guarded their crop field and chased monkey by shouting and following. Use of catapult and stone, use of dog to chase monkey, hitting tin boxes, as well as other method like radio and cassette players, fire crackers as well as planting alternative crops were also found. Khatry (2006) in Vijayapur, Dharan found the use of catapult to frighten the monkeys to be most effective.

Chalise (2001) reported that farmer's suffering from monkey crop damage in eastern Nepal was considering planting alteration of crop pattern. From the study, monkeys' unflavored crops would minimize the crop raiding problems. Ginger, garlic, chili, yam, and colocasia, etc. were the major alternative crops planted by the local people. People should give priority for alternative farming like mushroom cultivation, planting of chili, lady's finger, ginger, garlic etc. Alternative crop was found to be effective to lessen the loss by monkey. Local people were found to prefer worth compensation to lessen the conflict with monkey.

Acknowledgements

We are highly obliged to Prof. Dr. Ranjana Gupta, Head, Central Department of Zoology, Tribhuvan University, for her encouragement and valuable suggestions. Sincere thanks go to generous and supportive people of Arkhale and Nayagaun and VDCs staffs for their amicable behaviour in our research work as well as to all the respondents who spend their valuable time for providing necessary information pertinent to the study.

References

Chalise, M. K. 1997. Monkeys from Makalu-Barun Conservation Area (MBCA). NAHSON Bulletin. **7** (3-4): 4-9.

Chalise, M. K. 1999. Kasto Chha Bandar Haruko Bichitrako Sansar? (How wonderful is the Monkeys' world) in Nepali vernacular. Navayuba monthly, 4 yr. **33**: 1-20.

Chalise, M. K. 1999a . Crop raiding by wildlife, especially primates and indigenous practices for crop protection in Lakuwa area, east Nepal. Asian Primates. **7**: 4-9.

Chalise, M. K. 2001. Crop raiding by wildlife, especially primates and indigenous practices for crop protection in Lakuwa area, East Nepal. Asian Primates. **7**:4-9.

Chalise, M. K. 2003. Crop raiding by primates and other wild animals in the mountains of Nepal. Integrated pest management in Nepal. Neupane, F.P. (Ed.). Himalayan Resource Institute, Kathmandu. Pp. 155-63.

Chalise M. K. 2006. Primate census in different parts of Nepal. Journal of the University Campus TUTA, TU, Prospective on Higher Education. Vol. 2 & 3: 35-41.

Chalise M.K. 2010. A Study of Assamese Monkey in Sebrubeshi of Langtang National Park, Nepal. Journal of Natural History Museum, TU, Kathmandu, **25**: 54-61.

Chalise, M. K. and Johnson R.L. 2005.Farmers attitudes towards conservation of "pest" monkeys: the review from Nepal. In: J. D. Peterson and J. Wallis (Eds) USA, Commensalisms and Conflict: the human-primate interface. Special topics in primatology.American Society of Primatologists, Norman, Oklahoma, USA. **4**: 222-239

Khatry, P. 2006. Study on Monkey-Human conflict in Vijayapur area, Dharan, Eastern Nepal. M. Sc. Thesis, Tribhuvan University, Kathmandu, Nepal.

McCourt, P. 2005. Urban human-monkey conflict in the Vicinity of the Institute of Forestry, Hetauda, Nepal.

Roonwal, M. L. and Mohnot, S. M. 1977. Primates of South Asia, ecology, sociology and behavior.Harvard University Press, Cambridge, London, England.

Prevalence of *epizootic ulcerative syndrome* (EUS) in carps

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Abstract

In present study examination of fishes, present in 15 fish ponds, were done for the infestation of EUS in Chitwan, Nepal. Nine ponds or 60% of them were recorded infested and confirmed to be EUS positive. Among 1491 fish samples examined, 170 fish samples were found with lesions and 165 of them were confirmed to be EUS-positive. Two culture fish species like *Labeo rohita* and *Catla catla* was found to be containing lesions among different fish species. So, they were sampled for histological study. Other fish species like common carp, silver carp, grass carp and big head carp were not found to be EUS positive. The risk of EUS infestation was found high in fish ponds connected with paddy fields and flooded water supplying wild fish species. Ponds with poor management and use of contaminated nets also had a high risk of EUS infestation.

Keywords: *Labeo, Catla,* culture fish, lesions, pond management

Introduction

EUS is a fungal fish disease caused *Aphanomyces invadans* or *A. piscicida* belonging to family Saprolegniaceae. The disease has a complex infectious aetiology, clinically characterised by the presence of invasive *Aphanomyces* infection and necrotising ulcerative lesions. The early signs of the disease include loss of appetite, float on the surface and red spots on the body surface, head, operculum or caudal peduncle.

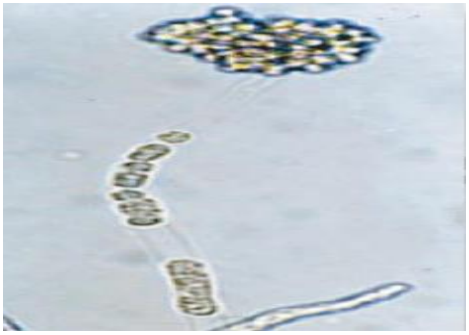

Plate 1. Aphanomyces

EUS is a highly infectious fish disease causing great loss in fish production specially tropical and sub tropical areas of South East Asia. Bangladesh suffered severe losses from EUS in 1988, 1989 and 1993. It was reported that 15-20% of total fish production loss in Nepal during initial EUS outbreaks (ADB/NACA 1991). Bhutan and the eastern Terai of Nepal were first affected in 1989. Later EUS was found to spread into Himalayan valley regions like Pokhara and Kathmandu in 1993 and cold water species (*Tor* spp) was also found to be infested (Phillips 1989). Pakistan was highly affected by EUS in snakeheads in Punjab Province in 1996 and in *Cirrhinus mrigala* in Sindh Province in 1998 (DFID 1998). Bhaumik et al. (1991) reported 73% of the culture ponds affected in West Bengal with about 30-40% fish loss.

Materials and Methods

General Field Surveys

The study was conducted in Shankar Chowk, Gujanagar VDC – 3, Chitwan from February 2012 to May 2012, recognized as EUS-season in Nepal. On arrival at the sampling pond site, information was gathered from the farmer and this was followed by the measurement of water quality parameters and examination of some susceptible fish. One or two fish of each species was sampled for histopathology study in the laboratory. Fish with lesion was killed and muscle of size $1cm^3$ taken from the lesion and surrounding for histology study. Samples were fixed immediately in 10% formalin and labeled. During the study period of 3 months, the randomly selected ponds were visited two times (15 days fortnightly).

Water quality parameters

Five important water quality parameters, i.e. Temperature, pH, Dissolved Oxygen, Alkalinity, Turbidity (Transparency), were measured fortnightly (15 days) two times in a

month in situ using portable water analysis kit (Hack kit) in 15 ponds. Water sample was collected between 7- 8 am from the surface (10 cm below).

- Dissolved Oxygen: Dissolved oxygen was measured fortnightly by using titration method.
- Alkalinity: Alkalinity was measured fortnightly by using titration method.
- Temperature: Temperature was measured fortnightly by using water thermometer-portable water analysis kit (Hack Kit) directly. Temperature was measured between 9- 10 am.
- pH: pH was measured fortnightly by pH meter in between 9- 10 am.
- Turbidity: Turbidity was measured fortnightly by using Secchi Disc (Boyd and Tucker 1992).

Histopathological analysis

Processing of formalin fixed tissues was carried out at Histopathological lab, Jana Maitri Hospital, Balaju, Kathmandu. First material was infiltrated with a medium that would give adequate support so that thin section (5-7µm) could be made by using Microtome laboratory (Chinabut and Roberts 1999).

Result

Occurrence of Ulcerative Lesions

Amongst the cultured fish species, rohu and naini (Indian major carps) were found to be affected by EUS. The total number of major carps examined was 1491. Among them, naini and rohu were comprised 1191 and 300 respectively. Of these, 170 were sampled with external lesions with histological damage and 165 of them were confirmed as EUS-positive based on the presence of mycotic granulomas. The average occurrence of EUS was 11.3 and 10.0 in naini and rohu respectively (Table 1).

Plate 2. Naini (*Cirrhinus mrigala*)

Plate 3. Rohu (*Labeo rohita*)

Table 1. Prevalence of EUS in carp pond fishes.

Name of Farmers	Fish Species	No. of fish examined			Confirm (EUS-positive)	Percentage (%)
		Total	Healthy	With Lesions		
Mr. Ghale	Naini	400	350	50	50	12.5
Mr. Badri	Rohu	300	265	35	30	10.0
Mr. Chaudhary	Naini	392	347	45	45	11.5
COA Group	Naini	399	359	40	40	10.0
	Total	1491	1321	170	165	Mean:11

Water quality and the occurrence of EUS

Aphanomyces invadans was found to grow best at 20–30°C but did not grow at 37°C. The *Aphanomyces* could transfer from one fish to another through the water supply. Both low and high temperatures appeared to influence outbreak. But low temperatures influenced the severity of EUS lesions and its outbreak.

Mis-management and the occurrence of EUS

The occurrence of EUS diseases was depended upon mismanagement of fish farms. The survey showed 45.5% of ponds in study areas did not have permanent source of water and

depended on other sources. Fish ponds that had water source from rice field, river, reservoir or wetland recorded relatively high incidence of EUS in comparison to ponds that had received water from underground source. The study showed 60% of the fishponds were not dried after harvest and remaining 40% of the ponds were recorded dried in the previous year only. About 36.8% of EUS occurrence was reported in ponds which were not dried in the previous year. 70% of the fish growers in study area were found not applying lime during pond drying and post stocking management period. All of the EUS positive confirmed cases were noted from the ponds that were not limed before.

Histological study

EUS disease can be confirmed by histological diagnosis. The early skin lesions of some samples was observed and found to be principally in the areas of epithelial necrosis with surrounding oedema, haemorrhaging of the underlying dermis and some inflammatory cell infiltration. The epidermis at the margins of the ulcer itself was degenerated and thickened due to the enclosing of a very small number of fungal hyphae within an epitheloid capsule. In advanced lesions, there is massive necrotising granulomatous mycosis of the underlying muscle fibres.

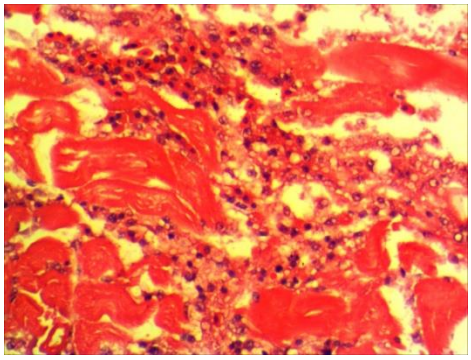

Plate 4. T.S. of skin of Rohu

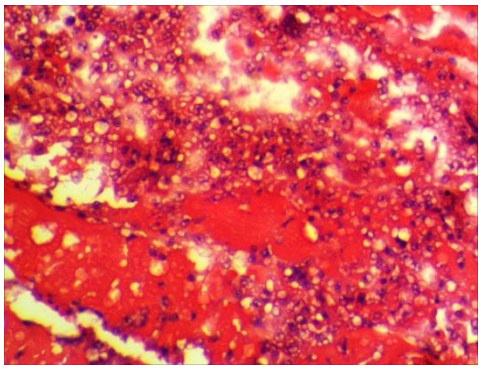

Plate 5. T.S. of skin of Naini

Discussion

EUS was a seasonal epizootic condition of great importance in freshwater carps. It was clinically characterized by the presence of invasive infection and necrotising ulcerative lesions. EUS is caused by the oomycete known as *Aphanomyces invadans* (Lilley et al. 2001b; Baldock et al. 2005; EFSA 2007, World Organisation for Animal Health OIE 2009).

Aphanomyces invadans could attach to the skin and invade underlying tissues. EUS outbreaks depended upon seasonal factors and water quality. *A. invadans* hyphae grew only poorly at temperatures above 31^{o}C and do not grow at 37^{o}C (Hatai et al. 1977; Fraser et al. 1992; Roberts et al. 1993).

The present study showed that 45.5% of ponds in Chitwan did not have permanent source of water and depended on rainfall. About 30 percent of ponds received water from the canal connected to the rice field while 20.0 percent received water from canal connected to river. Ponds that received water coming through rice field and river/ditch had showed high relative risk of EUS while the occurrences of this disease was less in ponds that had received water only from underground source. Similarly, ponds that were repeatedly flooded that year also showed a higher relative risk. Failure to drain and lime ponds prior to stocking has a high risk of outbreak of EUS (Mohan and Shankar 1994; Jha and Shrestha 2003).

References

Baldock F.C., Blazer V., Callinan R., Hatai K., Karunasagar I., Mohan C.V. and Bondad-Reantaso M.G. 2005 Outcomes of a short expert consultation on epizootic ulcerative syndrome (EUS): Re-examination of causal factors, case definition and nomenclature. In: Diseases in Asian Aquaculture V, Walker P., Laster R. & Bondad-Reantaso M.G., eds. Fish Health Section, Asian Fisheries Society, Manila, Philippines, 555–585.

Bhaumik, U., Pandit, P.K. and Chatterjee, J.G. 1991. Impact of epizootic ulcerative syndrome on the fish yield, consumption and trade in West Bengal. Journal of the Inland Fisheries Society of India **23**(1): 45-51.

EFSA, 2007. Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on possible vector species and live stages of susceptible species not transmitting disease as regards certain fish diseases. The EFSA Journal 584, 1-163.

EFSA, 2008. Scientific Opinion of the Panel on AHAW on a request from the European Commission on aquatic animal species susceptible to diseases listed in the Council Directive 2006/88/EC. The EFSA Journal 808, 1-144.

Fraser G.C., Callinan R.B. and Calder L.M. 1992. *Aphanomyces* species associated with red spot disease: an ulcerative disease of estuarine fish from eastern Australia. J. Fish Dis., **15**: 173–181.

Hatai K., Egusa S., Takahashi S. & Ooe K. 1977. Study on the pathogenic fungus of mycotic granulomatosis – I. Isolation and pathogenicity of the fungus from cultured-ayu infected with the disease. Fish Pathol. **12**: 129–133.

Jha, D.K. and Shrestha, M.K. 2003. Occurrence of Epizootic Ulcerative Syndrome in Chitwan, p.54. In Book of Abstract. International Conference on Disease Management for Sustainable Fisheries held in Trivandram, India.25-28 August, 2003.

Lilley, J.H., S. Chinabut, and J. C. Miles, 2001b. Applied Studies on Epizootic Ulcerative Syndrome. Institute of Aquaculture, University of Stirling, Scotland; Aquatic Animal Health Research Institute, Department of Fisheries, Thailand, Stirling.

World Organisation for Animal Health OIE, 2009. Manual of Diagnostic Tests for Aquatic Animals, OIE, Paris,

Philips, M.J. 1989. A report on the ACA workshop on the regional research programme on ulcerative syndrome in fish and the environment, 20-24 March 1989. Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand.

Roberts, R. J., Willoughby, L. G. and Chinabut, S. 1993. Mycotic aspects of Epizootic Ulcerative Syndrome (EUS) of Asian fishes. J. Fish. Dis. **16** (3): 169-183.

Prevalence of intestinal helminth parasites among school-children of Bhaktapur district, Nepal

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Abstract

The present study has been carried out on school children of Everest English School and Prabhat English Higher Secondary School of Bhaktapur District, Nepal from June 2011 to March 2012 in order to determine the prevalence of intestinal helminthes. A total of 495 stool samples from the children of two schools were examined by direct smear method under microscope. Among them, 137 (27.67%) were found positive for one or more intestinal helminth parasites. *Ascaris lumbricoides* (22.63%) showed highest prevalence followed by *Trichuris trichiura* (6.06%), *Strongyloides stercoralis* (1.82%), Hookworm (1.62%), *Taenia* sp. (1.01%), *Hymenolepis nana* (0.81%) and *Enterobius vermicularis* (0.40%). The helminthic infection was found to be almost equal in male and female children and statistically no significant difference was found ($\chi^2 = 8.31\times10^{-6}$, $p>0.05$). Out of 137 positive cases, 78.83% were infected with single parasites. Double and multiple infections were found among 18.98% and 2.19% of students respectively. The occurrence of intestinal helminth parasites may be attributed to unhygienic way of life, poverty, ignorance and poor sanitation.

***Keywords*:** *Ascaris*, Hookworm, Prevalence, School children, *Trichuris*.

Introduction

Nepal is a nation full of ancient glories rich in tradition, culture and civilization. Nepal exhibits social, ethnical, linguistic and cultural diversity. Life in Nepal, like in most of the third world countries is characterized by poverty, ignorance and diseases. Intestinal infestations like giardiasis, amoebiasis, ascariasis, ancylostomiasis, fascioliasis and taeniasis were common in Nepal (Acharya 1979). Children were found to be infected more frequently by intestinal parasites than adults (Rai et al. 1994). Intestinal parasitic infections are major

causes of morbidity and mortality among school aged children of developing countries (WHO 1987). School-aged children and preschool children are the most vulnerable group as compared with any other age group, and they harbour the greatest numbers of intestinal worms. As a result, they experience growth stunting and diminished physical fitness as well as impaired memory and cognition (Stephenson et al. 2000, Crompton and Nesheim 2002, Bethony et al. 2006, Tchuem Tchuenté 2011). The public health importance of intestinal parasitoses continues because of its high prevalence, virtually global distribution and effects on both nutritional and immune status of individuals (WHO 1987). Over 270 million preschool-age children and over 600 million school-age children live in areas where soil-transmitted helminth (STH) parasites are intensively transmitted, and are in need of treatment and preventive interventions (WHO 2012). The relative importance of the major groups of helminthes may be roughly judged by Stoll's (1947) estimate that explains among 2200 million people, 72 million cestodes, 148 million trematodes and over 2000 million nematodes are present (Stoll 1947, Chandler and Read 1961). Fifty years after Stoll published his 'This Wormy World' article, the global prevalence of infections with intestinal nematodes remains virtually unchanged (Chan 1997).

Materials and Methods

Bhaktapur is divided into two municipalities and 16 Village Development Committees (VDCs) for administrative purpose. The two municipalities include Madhyapur Thimi and Bhaktapur municipality among which latter was chosen as study area. The total population of Bhaktapur municipality is 72,543 with 12,395 households according to census 2001 (CBS, Nepal 2001). Two schools: Everest English School (EES) (in ward no. 15) and Prabhat English Higher Secondary School (PEHSS) (in ward no.10) were purposively selected for the study. The study was designed to include the school-children of the age group 9-12 years. According to the school records, school children of this particular age group belonged to classes 4, 5 and 6. Hence, all students from class 4, 5 and 6 of these two schools were decided to include as sample population. Out of 585 students of target population (358 from EES and 227 from PEHSS), stool samples from altogether 495 students (295 from EES and 200 from PEHSS) were collected. Stool samples were transported to parasitology laboratory

of Central Department of Zoology, Kirtipur, Kathmandu and examined by direct smear method for detection of intestinal helminth parasites. Macroscopic and microscopic examinations were conducted for laboratory diagnosis of helminth parasites in the collected stool samples.

Result

Out of 495 stool samples examined, 137 (27.67%) were found to be infected with one or many types of intestinal helminth parasites. The male and female children accounted for 58.39% and 41.60% of total positive cases (i.e., 137). Statistically, no significant difference was found in prevalence of intestinal helminth parasites in male and female ($\chi^2 = 8.31\times10^{-6}$, $p>0.05$).

School-wise prevalence of intestinal helminth parasites

Among 295 stool samples collected from Everest English School, 85 (28.81%) school children were infected with at least one of the intestinal helminth parasites. Likewise, 200 stool samples were collected from Prabhat English Higher Secondary School of which 52 (26.00%) school children were found to be infected. The rate of infection was observed to be slightly higher in Everest English School than in Prabhat English Higher Secondary School. Statistically, no significant difference was found in school-wise prevalence of intestinal helminth parasites ($\chi^2 = 0.471$, $p>0.05$).

Prevalence of intestinal helminth parasites

The study revealed that *Ascaris lumbricoides* was most common intestinal helminth parasite with a prevalence rate of 22.63% (112/495). *Enterobius vermicularis* was the least prevalent helminth parasite with prevalence rate of 0.40% (2/495). 30 (6.06%) students were infected with *Trichuris trichiura,* 9 (1.82%) with *Strongyloides stercoralis*, 8 (1.62%) with Hookworm, 5 (1.01%) with *Taenia* sp. and 4 (0.81%) with *Hymenolepis nana*. Among 137 positive cases, *A. lumbricoides* accounted for 81.75%, *T. trichiura* 21.89%, *S. stercoralis* 6.57%, *Taenia* sp. 3.65%, Hookworm 5.84%, *H. nana* 2.92% and *E. vermicularis* 1.46% (Figure 1).

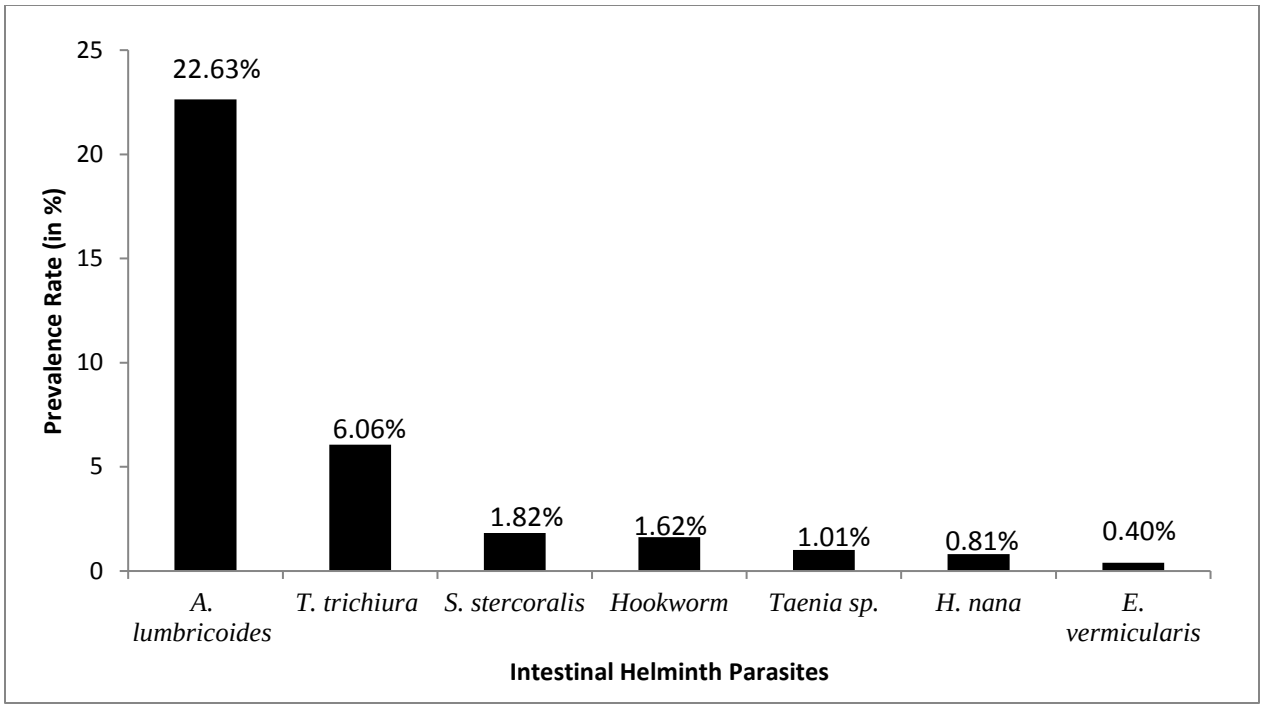

Figure 1. Prevalence of different species of intestinal helminth parasites

Concurrent helminth infection among school children

Infection with single helminth parasite in an individual was more common than double or multiple infections. Out of 137 positive cases, 108 cases (78.83%) included infection with single parasite, 26 (18.98%) were infected by double helminth parasites and 3 (2.19%) were infected by multiple species of helminth parasites. Regarding double infection, co-infection of *T. trichiura* with *A. lumbricoides* was found to be most prevalent i.e., 14 (56.00%). In multiple infections, one case each of co-infection of *Ascaris*, Hookworm and *Trichuris*, co-infection of *Ascaris*, *Enterobius* and *Trichuris* and co-infection of *S. stercoralis* with Hookworm, *A. lumbricoides* and *T. trichiura* were observed.

School, Sex and Species-wise prevalence of intestinal helminth parasites

A. lumbricoides was most prevalent helminth parasite in both male (21.10%) and female (24.76%) children. *E. vermicularis* was least prevalent among male children (0.69%) while *E. vermicularis* and *H. nana* were completely absent among female children (0.00%). The prevalence rates of infection with *T. trichiura* were 7.27% and 4.37%, *Taenia* sp. were 1.38% and 0.49%, *S. stercoralis* were 2.08% and 1.46%, Hookworm were 2.08% and 0.97% and *H.*

nana were 1.38% and 0.00% in male and female school children respectively. The prevalence of *A. lumbricoides* was highest in both the schools under study. Among 295 students in Everest School, 70 (23.73%) were infected with *A. lumbricoides* followed by 14 (4.75%) infected with *T. trichiura*, 5 (1.69%) with *Taenia* sp., 4 (1.36%) with *S. stercoralis*, 3 (1.02%) with Hookworm, 2 (0.68%) with *E. vermicularis* and 1 (0.34%) with *H. nana*. Among 200 students from Prabhat School, 42 (21.00%) were infected with *A. lumbricoides* followed by 16 (8.00%) infected with *T. trichiura*, 5 (2.50%) with *S. stercoralis*, 5 (2.50%) with Hookworm and 3 (1.50%) with *H. nana.* However, *Taenia* sp. and *E. vermicularis* were not recorded (Table 1).

Table 1. School, Sex and Species- wise prevalence of intestinal helminth parasites

Name of Parasite	Everest English School				Prabhat English School				Grand Total (N=495)	Prevalence %
	Male (n=179)	Female (n=116)	Total (N=295)	Prevalence %	Male (n=110)	Female (n=90)	Total (N=200)	Prevalence %		
Ascaris	38	32	70	23.73	23	19	42	21.10	112	22.63
Trichuris	9	5	14	4.75	12	4	16	8.00	30	6.06
Taenia sp.	4	1	5	1.69	-	-	-	-	5	0.81
E. vermicularis	2	-	2	0.68	-	-	-	-	2	0.40
S. stercoralis	2	2	4	1.36	4	1	5	2.50	9	1.82
Hookworm	2	1	3	1.02	4	1	5	2.50	8	1.62
H. nana	1	-	1	0.34	3	-	3	1.50	4	0.81
Total	58	41	99		46	25	71		170	

Discussion and Conclusion

Intestinal parasites are worldwide in distribution. Among them Soil Transmitted Helminthes (STHs) and other helminth parasites pose serious threat in the physical well-being of human. Poverty, illiteracy and different aspects of culture may play leading role in increasing rate of prevalence of such parasites.

The present study indicated that the prevalence of intestinal helminthes in school children was remarkable. Out of 495 children, 137 (27.67%) were found to be infected by at least one type of intestinal helminthes. This high rate of prevalence among school children may be associated with unsanitary living style, poor socio-economic conditions, usual contact with soil and consumption of vegetables, fruits and water contaminated with infected faeces which was not considered in this study. Overall prevalence rate among school children showed comparatively less than several studies which showed higher rate than 50% (Sugari et al. 1985, Gupta and Gupta 1988, Sharma et al. 2004). Comparable prevalences of helminthes were, however, reported in some other studies (Jha 2004, Shakya et al. 2006, Mukhopadhyay et al. 2007). Rai et al. (1994) concluded that the annual rate of positivity for STH ranged from 18.0-36.6%.

Present findings showed that the rate of prevalence is independent of the sex of children (χ^2= 8.31×10^{-6}, $p>0.05$). This may be due to the reason that children from both sexes share similar type of environment and feeding habit and hence are equally susceptible for transmission of helminth parasites. Similar findings have also been reported previously (Rai et al. 2002, Manandhar 2007).

In this study seven different types of helminth parasites were found which were: *Ascaris lumbricoides* (22.63%), *Trichuris trichiura* (6.06%), *Strongyloides stercoralis* (1.82%), Hookworm (1.62%), *Taenia* sp. (1.01%), *Hymenolepis nana* (0.81%) and *Enterobius vermicularis* (0.40%). These parasites had also been reported from rural area of Kirtipur (Chaudhari 2004), from rural areas of Southern Nepal (Sherchand et al. 1997) and from Chepang children of Taklung, Gorkha (Pokhrel 2005).

Several previous studies have shown that Hookworm was the most prevalent helminth parasite in Nepal (Estevez et al. 1983, Sherchand et al. 1997, Yong et al. 2000, Kunwar et al. 2006). Similarly, some other studies have shown *Trichuris trichiura* as the most common helminth (Shrestha 1983 in Panchkhal, Uga et al. 2004, Pokhrel 2005 in Gorkha, Rai et al. 2005). However, the present study has shown that *A. lumbricoides* (22.63%) is the most prevalent intestinal helminth parasite followed by *T. trichiura* (6.06%). This result is in agreement with that reported previously (Gupta and Gupta 1988, Chhetri 1997, Manandhar 2007, Sukupayo 2007) which also showed *A. lumbricoides* as most prevalent helminth in Nepal followed by *T. trichiura*. Sharma (1965) showed Roundworm infection was common

in Bhaktapur. Comparable results were also obtained from Jha (2004) with *A. lumbricoides* (23.06%) the commonest helminth among adolescent girls of Kirtipur, Maharjan (2005) and Kunwar et al. (2006). Williams-Blangero et al. (1993) concluded that Roundworm, Whipworm and Hookworm were endemic in Nepal and were the major health problem for the population which seems to be still true. The high prevalence of soil transmitted helminthes (*A. lumbricoides, T. trichiura, S. stercoralis* and Hookworm) among helminthes has been increasingly recognized by WHO as an important public health problem, particularly in developing countries. Gurbacharya (1981) observed STH infection in Bhaktapur was higher than other types of parasites.

Among two schools where studies were conducted, the prevalence rate showed no statistical difference (χ^2 = 0.471, p>0.05) although higher rate was found in Everest English School (28.81%) than in Prabhat English Higher Secondary School (26.00%). This could be due to the dispersal of parasites in Bhaktapur allowed equal chance of infection to all particular age-grouped children.

Out of 137 positive cases, highest percentage was found for infection with single helminth (78.83%) followed by double infection (18.98%) and multiple infection (2.19%). The most common double infection was that of *A. lumbricoides* plus *T. trichiura* i.e., 10.22% of total positive cases. Previous studies had also shown similar results (Pokhrel 2005, Manandhar 2007). Just two individuals were infected by three species of helminthes (*Ascaris* + *Enterobius* + *Trichuris* and *Ascaris* + Hookworm + *Trichuris)* and only one individual has quadruple infection (*Ascaris* + *Trichuris* + Hookworm + *Strongyloides*). These results also indicated that *Ascaris* was highly distributed among school children followed by *Trichuris*. This finding is also supported by Rai et al. (1994) who reported that the annual rate of the positivity for soil transmitted helminthiasis had the highest prevalence rate than others.

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References

Acharya, S. 1979. Malnutrition and diarrhoeal disease. Journal of Institute of Medicine **1**: 21-23.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D. et al. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. The Lancet **367**(9521): 1521–1532.

CBS, Nepal 2001. Central Bureau of Statistics, Nepal (web).

Chan, M.S. 1997. The global burden of intestinal nematode infections–fifty years on. Parasitology Today **13**(11): 438–443.

Chandler, A.C. and Read, C.P. 1961. Introduction of Parasitology. John Wiley and Sons, Inc. New York, London.

Chaudhari, B., Mishra, P.N. and Sherchand, J.B. 2004. Prevalence of Human Intestinal Parasites in rural village development committee, Machchhegaun, Kathmandu. Fourth National Conference on Science and Technology, 23-26 March 2004. Royal Nepal Academy of Science and Technology, Khumaltar, Lalitpur SSZ-Ps8: 333.

Chhetri, M.K. 1997. Parasitic Infection in Nepal. Journal of Nepal Medical Association. **35**: 60-65.

Crompton, D.W.T. and Nesheim, M.C. 2002. Nutritional impact of intestinal helminthiasis during the human life cycle. Annual Review of Nutrition **22**: 35–59.

Estevez, E.G., Levine, J.A., and Warren, J. 1983. Intestinal parasites in a remote village in Nepal. Journal of Clinical Microbiology **17**(1): 160-161.

Gupta, R. and Gupta, H.N. 1988. Studies on the Infestation Rate of Human Intestinal Parasites of Kirtipur. Journal of Nepal Medical Association **26**(4): 23-29.

Gurbacharya, D.L. 1981. Problem of Soil Transmitted Helminthiasis in Nepal. Journal of Nepal Medical Association **19**(4).

Jha, A. 2004. Prevalence of intestinal parasites in adolescent girls in relation to socio-behavioural aspects in Kirtipur Municipality. M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Kunwar, C.B., Chapagain, R.H., Subba, B., Shrestha, M., Jha, B., Subedi, J. et al. 2006. Occurrence of soil-transmitted helminths in women at the Himalayan region of Nepal. Kathmandu University Medical Journal (KUMJ) **4**(4): 444-447.

Maharjan, K.P. 2005. Prevalence of intestinal parasites in children of Kirtipur area with respect to their socio-cultural and socio-economic status. M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Manandhar, P. 2007. Intestinal parasitic infections among the children of Ganesh Secondary School of Bhaktapur District. M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Mukhopadhyay, C., Wilson, G., Pradhan, D. and Shivananda P.G. 2007. Intestinal protozoal infestation profile in persistent diarrhea in children below age 5 years in western Nepal. The Southeast Asian Journal of Tropical Medicine and Public Health **38**(1): 13-19.

Pokhrel, Y.B. 2005. Epidemiology and control strategy of intestinal parasites among Chepang children (<16 years) at Taklung VDC of Gorkha. M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Rai, D.R., Rai, S.K., Sharma, B.K., Ghimire, P. and Bhatta, D.R. 2005. Factors associated with intestinal parasitic infection among school children in a rural area of Kathmandu Valley, Nepal. Nepal Medical College Journal **7**(1): 43-46.

Rai, S.K., Hirai, K., Abe, A., Ishiyama, S., Rai, G., Ono, K. et al. 2002. Intestinal parasitosis among school children in a rural hilly area of Dhading district, Nepal. Nepal Medical College Journal **4**: 54-58.

Rai, S.K., Kubo, T., Nakanishi, M., Sumi, K., Shibata, H., Matsuoka, A. et al. 1994. Status of soil-transmitted helminthic infection in Nepal. The Journal of the Japanese Association for Infectious Diseases **68**(5): 625-630.

Shakya, B., Rai, S.K., Singh, A. and Shrestha, A. 2006. Intestinal parasitosis among the elderly people in Kathmandu Valley. Nepal Medical College Journal **8**(4): 243-247.

Sharma, B.K., Rai, S.K., Rai, D.R. and Choudhury, D.R. 2004. Prevalence of intestinal parasitic infestation in schoolchildren in the northeastern part of Kathmandu Valley, Nepal. The Southeast Asian Journal of Tropical Medicine and Public Health **35**(3): 501-505.

Sharma, B.P. 1965. Roundworms and other infestations. Journal of Nepal Medical Association **3**(2): 120-123.

Sherchand, J.B., Ohara, H., Sherchand, S., Cross, J.H. and Shrestha, M.P. 1997. Intestinal Parasitic Infection in Rural Areas of Southern Nepal. Journal of Institute of Medicine **19**(3-4): 115-121.

Shrestha, I.L. 1983. Soil transmitted helminthiasis. Journal of Institute of Medicine **5**(2): 193-196.

Stephenson, L.S., Latham, M.C. and Ottesen, E.A. 2000. Malnutrition and parasitic helminth infections. Parasitology **121**(Supplement): S23-38.

Stoll, N. 1947. This Wormy World. Journal of Parasitology **33**: 1-18.

Sugari, S., Toongu, Y., Inatomi, S. and Pradhan H.D. 1985. A survey on human parasite infection in Nepal. Japanese Journal of Parasitology **34**: 285-291.

Sukupayo, P.R. 2007. Prevalence of intestinal helminth parasites among students of Dyola School of Bhaktapur. M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Tchuem Tchuenté L.A. 2011. Control of soil-transmitted helminths in sub-Saharan Africa: Diagnosis, drug efficacy concerns and challenges. Acta Tropica **120** Supplement 1:S4-S11.

Uga, S., Rai, S.K., Kimura, K., Rai, G., Kimura, D., Wakasugi, M. et al. 2004. Parasites detected from diarrheal stool samples collected in Nepal. The Southeast Asian Journal of Tropical Medicine and Public Health **35**(1): 19-23.

WHO 1987. Prevention and Control of Intestinal Parasitic Infections: Report of a WHO Expert Committee. WHO Technical Report Series: 749, World Health Organization, Geneva.

WHO 2012. Soil-transmitted helminth infections. http://www.who.int/mediacentre/factsheets/fs366/en/. Accessed on 10 August, 2012.

Williams-Blangero, S., Adhikari, B.N., Blangero, J., Robinson, E.S., Upreti, R.P. and Pyakurel, S. 1993. Helminthic infection in Jiri, Nepal: Analysis of age and ethnic group effects. Journal of Institute of Medicine **15**: 210-216.

Yong, T.S., Sim, S., Lee, J., Ohrr, H., Kim, M.H. and Kim, H. 2000. A small-scale survey on the status of intestinal parasite infections in rural villages in Nepal. Korean Journal of Parasitology **38**(4): 275-277.

Predatory insects of *Adelges tsugae* Annand (Homoptera: Adelgidae) at Dhungkharka, Kavrepalanchok, Nepal

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Abstract

Predatory insects of *Adelges tsugae* Annand (Homoptera: Adelgidae) were studied in Dhungkharka, Kavrepalanchok. The study also assessed their seasonal abundance with respect to *A. tsugae* infestation and their feeding efficacy. Host plant selected for the study was hemlock plant. The predatory insects were collected by sweaping and beating. Two insects viz. *Syrphus* sp. (Syrphidae) and *Coccinella septempunctata* L. (Coccinellidae) were recorded as predators. *Coccinella septempunctata* was found more effective to control *A. tsugae*. The highest density of predators was recorded during May (8.9/branch) and June (9.2/branch) corresponding to abundance of pests. Result showed positive correlation between predator and pest population ($p<0.05$). Mean number of the pest consumed by male *C. septempunctata* (12.67±3.47 SD) was found less than that by female (16.87±4.39 SD). The difference in consumption capacity is statistically significant ($p<0.05$).

Key words: Hemlock, *Coccinella*, *Syrphus*, infestation, pest

Introduction

Hemlock Woolly Adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) is parthenogenic aphid-like insect feeding on several species of *Tsuga* and *Picea* (Pinaceae). *Adelges tsugae* is dispersed by wind, birds, deer, and humans, and is moving north at a rate of about 30 km/year (McClure 1990). The most obvious symptom of infestation is the presence of white woolly egg sacs on the underside of hemlock needle, especially on new growth. Once established on a host, the adelgids feed on twigs of new growth by piercing at the base of needle (Young et al. 1995). Shortly after infestation, the feeding causes the desiccation of the needles and eventually results in the death and falling to the ground. Infested tree usually die

within 9 years, but the trees have been killed in as little as 4 years in case of heavy infestation (McClure 1987). The adelgid completes two generations per year each on host plants *Picea* and *Tsuga* (McClure 200l) and population grows very rapidly. Two generations develop simultaneously in the spring: winged (sexuparae) and non-winged (progrediens). Sexuparae developed into adult and it leaves hemlock in search of a *Picea* for oviposition. Progrediens develop during autumn and winter, following a summer aestivation. Nymphs of both types hatched in April (McClure et al. 2001).

Hemlock is a valuable timber species, it is used widely for pulp wood and for building barns, sheds and other structures. Canada balsam is obtained form eastern hemlock, *Tsuga canadensis*. It is commonly used as mounting medium for microscope preparation as its refractive index is approximately equivalent to that of glass (Sharma 1995). Hemlock spruce oil is essential oil which is extracted from hemlock and spruce plants. A number of products like rayon, transparent films, photographic films, artificial sponges and licquers are made from the wood pulp of *Picea* and *Tsuga* species. Hemlocks are long-lived, late successional climax trees that, if left undisturbed, eventually dominant stands. Its species occupies an important ecological niche. Hemlock stands provide cover for grouse, deer, snow-shoes hare and rabbit. Many birds use hemlock as a food source, nesting site etc.

Many species of predatory insects were reported from different parts of Asia and North America, but none of them were effective biological control agents to maintain hemlock woolly adelgid population below the economic injury level. 80 species of insects and 12 species of mites have been recorded as predators of *Adelges* from Himalayas (Rao and Ghani 1972), which includes the families; Anthocoridae: Hemiptera, Syrphidae: Diptera, Chamaemyidae: Diptera, Chrysopidae: Neuroptera, Hemerobidae: Neuroptera, Coccinellidae: Coleoptera and Acarina. The most significant natural predator of hemlock woolly adelgid is *Pseudoscymnus tsugae* (Coccinellidae:Coleoptera) which has been most effective predator in Japan and is mainly responsible for checking hemlock woolly adelgid population (Sasaji and McClure 1997). The oribatid mite, *Diapterobates humeralis* Hermann (Oribatida: Cerotozetidae) is especially effective in locating and destroying the cottony sacs which covers the eggs of adelgid in Japan (McClure 1995). *Laricobius nigrinus* (Derodontidae: Coleoptera) larvae primarily feed on eggs while adults feed on all stages, and

it has been evaluated as a potential biological control agent of hemlock woolly adelgid (Salom et al. 1999).

Heavy infestation undoubtedly causes progressive weakening of the trees and makes them more susceptible to disease. Chemical control options available for use in landscaped settings are inappropriate for treating hemlocks in forest settings (McClure et al. 2001). Because stands are scattered, full insecticides coverage is hard to achieve, and the trees are often located in sensitive areas like near the streams. Current study on hemlock woolly adelgid is focusing on the identification of natural predators which will remain viable and effective at regulating hemlock woolly adelgid population in native home or in the outside world. Moreover the study deals with seasonal abundance of predators in relation to pest infestation and the feeding behavior of predators.

Materials and Methods

Study area

The study was carried out from April to August 2002 at Dhungkharka, Kavrepalanchok of central Nepal (85^0 30' 15" to 85^0 30' 45" E and 27^0 30' 15" to 27^0 30' 45" N). It is situated at 2400 m asl with sub-tropical climate and comprised temperate and alpine conifer forests. The site is a dominant himalayan hemlock forest but some other mixed hardwood trees (*Rhododendron*, *Pinus roxburgii, Pinus wallichii, Lyonia ovalifolia, Castonopsis*, etc) are also found. A few ephemeral streams were there in the beginning of the study as it was started in April, the semi-hot spring and they were about to be dried. Abundant hemlocks were infested by the *Adelges tsugae* but the infestation was not so severe to cause death.

Preliminary Field Survey

A preliminary field survey was conducted to locate the infestation of *A. tsugae* on hemlock plant in and around the main study area before the actual field work was started.

Field Setting

Three sites in Narayanthan community forests were selected for the study. All study sites were along north-east facing slope between 2100 to 2300 m. Five trees of *Tsuga dumosa* (about 3 to 4.5 m in height) were randomly selected from each site. Observation of pest and predators was made at an interval of 15 days for 5 months. Each tree was divided into upper,

middle and lower crowns to draw samples from three levels. The twigs from top, middle and lower crown were drawn. From each region, a twig is drawn from the area of 20 x 20 cm^2 (0.04 m^2) and the hemlock woolly adelgids settled in each sample crown were counted and recorded. Total sampled branch per tree was 4.5 sq. m. Only egg masses were taken into account in case the adults were missing due to disturbances.

Sweeping

Estimation of active flier predatory insects was made by sweeping of insect net (handle: 150 cm long hollow steel rod, steel wire ream: 30 cm diameter, bag: muslin cloth of 1 mm^2 mesh size and about thrice as long as the diameter of the ream). In each sample, sweeping was done around the sampled branch five times for the collection of insects. The captured predatory insects were counted and recorded for each sample.

Beating

The sampled branch was beaten by a stick for the estimation of predaceous beetles and other predatory crawler insects per branch following Oldroyd (1958). A white cotton cloth (2 x 2 m.) was stretched and placed under a tree. The predatory insects dropped on cloth were counted and summed it with total number of predators recorded in 3 crown samples of tree. Some infested twigs along with crawler of *A. tsugae* and predators (using mailing tube) were brought to the laboratory in live condition.

Laboratory Setting

Feeding behavior of the predatory beetle was studied in laboratory. The predators were kept in the petri-dishes (10 x 15 cm) with moistened filter paper at the bottom. The filter papers were replaced daily by new ones to avoid contamination. Male and female adult predatory beetles, maintained in different petri-dishes, were provided with 30 adelgids per day for feeding. The adelgids were replaced every morning by fresh ones. The number of adelgids consumed per day and that left uneaten were every morning.

Identification

The predatory insects were identified following Coccinelids of Nepal (Kapur 1958) and Fauna of British India (Brunetti 1923). The identification was verified by comparing with the specimens at Natural History Museum, Swoyambhu, Kathmandu and Nepal Agricultural Research Council, Khumaltar, Lalitpur.

Data Analysis

The data were analyzed using various statistical tools like mean, correlation and t-test following Flower et al. (1998).

Results

Predatory Insects of Adelgids

Adelges tsugae infestation was noticed from April to August, 2002. Only one species of Coccinellids predatory beetle, *Coccinella septumpunctata* L. and one genera of syrphids, *Syrphus* spp. were recorded during the study period. *Syrphus* spp. was observed in the beginning of the study, but relatively less in number; 0.6/branch in April, 0.4/branch in May and 0.1/branch in June (Figure 1). It was not noted in July and August. The high population density of *C. septumpunctata* was recorded in May (8.5 per branch) and June (9.1 per branch), and least in August (1.4 per branch) (Figure 1).

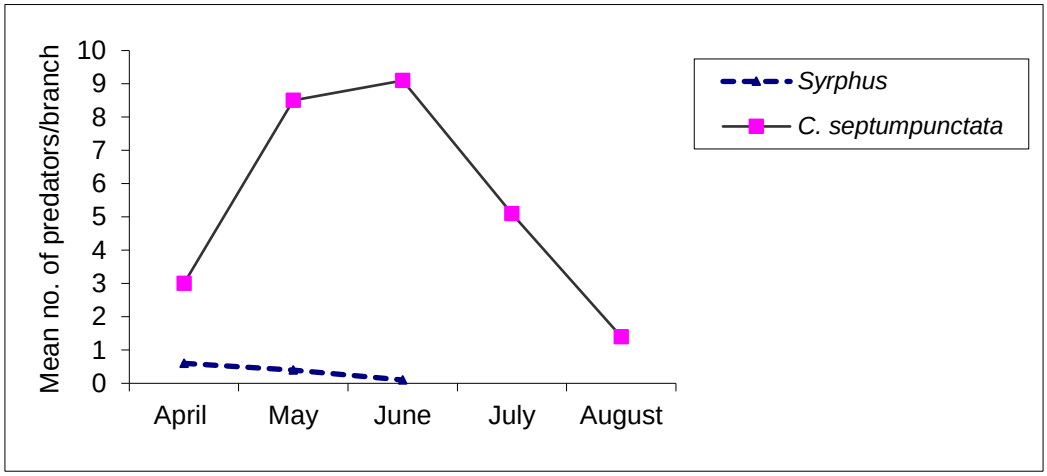

Figure 1. Abundance of predatory insects in late spring and summer.

Seasonal Abundance of Pest and Predator

Population of predator and prey was inconsistent in different seasons. *Adelges tsugae* population was maximum in early-summer (1060/branch) and the population decreased form mid-summer and recorded least in early-autumn (242.5/branch) (Table 1). Similarly, the maximum number of predators was recorded in early summer (8.9/branch) and mid summer (9.2/branch) while the lowest in early autumn (1.4/branch) (Table 1).

Table 1. Seasonal Abundance of pest and predator per branch.

Month	Total pop^{n} of *A. tsugae*	Mean No. of *C. septumpunctata*	Mean no. of *Syrphus* spp.	Total pop^{n}. of predators
April	880.0	3.0	0.6	3.6
May	1060.0	8.5	0.4	8.9
June	665.0	9.1	0.1	9.2
July	465.0	5.1	-	5.1
August	242.5	1.4	-	1.4

Feeding Potential of *C. septumpunctata*

The feeding efficiency of male and female of *C. septumpunctata* was studied separately in the laboratory. The result showed dissimilar feeding potential in male and female. Average number of A. tsugae consumed per day was 12.67±3.47 and 16.87±4.39 for male and female repectively (Figure 2). Difference in feeding efficacy of male and female was found to be statistically significant ($p<0.05$).

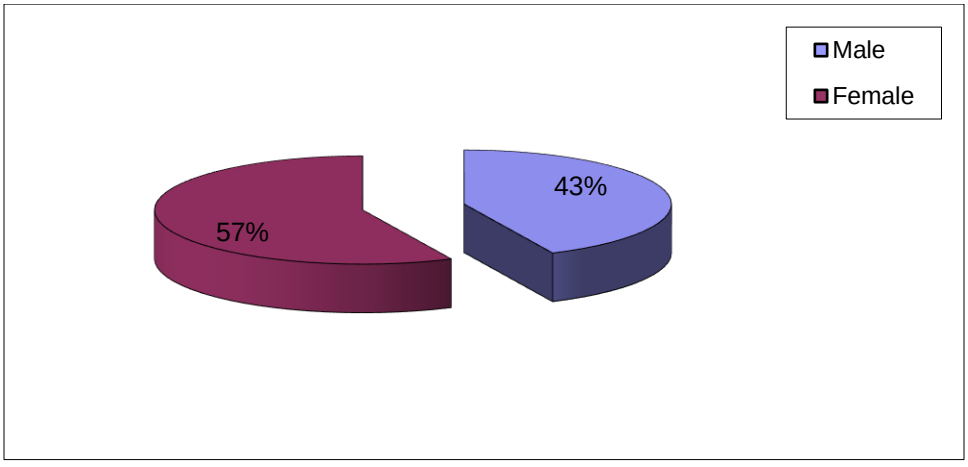

Figure 3. Feeding capacity of male and female *C. septumpunctata* in percentage

Discussion

Only two species of adults predatory insects were found in association with *Adelges* in Dhungharka. These species are *Syrphus* sp. and *C. septumtunctata*. *Coccinella septumpunctata* feeding on *Adelges* spp. observed by Laidlaw (Nagarkatti and Ghani 1972) in Scotland. Among two recorded predators, *Syrphus* spp. is evaluated as less important predator because it was recorded less in numbers, 0.6, 0.4 and 0.1 per branch from April to June respectively. Whereas the number of *C. septumpunctata* was found to be ranged form 1.4–9.1 per branch during the study period (Figure 1). Only the larval stages of *Syrphus* feed upon *Adelges* but both larval and adult stages of *C. septumpunctata* are the effective predators of *Adelges*. These findings are similar to those of Rao and Ghani (1972) who reported syrphids as the less effective predator than coccinellids.

Abundance of predators with *A. tsugae* infestation was different in different months. An infestation of hemlock woolly adelgid was noticed maximum in May with 1060 adelgids per branch and minimum in August with 242.5 per branch (Table 1). A steady decrease in *A. tsugae* population was observed from May to August. The steady reduction in population is due to absence of spruce plant in forest as the sexuparae adults oviposit in the spruce plant and progrediens generation go for summer aestivation. McClure (1991 and 2001) also reported the adults of sexuparae generation oviposit in the spruce plant and progrediens generation go for summer aestivation.

Mean population of all predators reported during the study period ranged from 1.4 to 9.2. The maximum number of predators noted in May–June and minimum number in August which coincide with the out break of *A. tsugae* population in May. The correlation analysis of number of pest and predators showed that the predator population was positively correlated with the number of pests. Thus it indicates the predators' (*C. septumpunctata* and *Syrphus* sp.) population increases with the increase in hemlock woolly adelgid population in different months. These findings are similar to those of Nagarkatti and Ghani (1972) who reported the appearance of *C. septumpunctata* in large numbers synchronising with increase of *Adelges* population in early or mid spring in Dalhousie region.

Present study showed that the female beetles consumed more *Adelges tsugae* (16.866±4.39/day) than male (12.666±3.47/day) during the adult stage. The number of

Adelges consumed by the female and male ranged from 9 to 25 and 6 to 18 respectively. The difference in the consumption capacities of the male and female is statistically significant ($p>0.05$). Kakkar et al. (2000) also reported the different consumption capacities of male and female and in their study, the adult male and female *C. septumpunctata* consumed 95.04 and 122.44 cereal aphid, *Sitobion avenae* respectively. Singh and Malhotra (1979) also reported the difference in consumption of aphid per day by male (95) and female (110).

Adelges tsugae attain high population density in May on Himalayan hemlock but not a single plant was gone to be threatened by the infestation of hemlock woolly adelgid in Dhungkharka. The population of pest was kept in check by the natural enemies like *C. septumpunctata* and *Syrphus* larvae and may also be due to the other factors like other predators not observed during the study period and plant resistance. In Asia, hemlock woolly adelgid number are kept in check by the presence of natural enemies and by host plant resistance (McClure 1995; Sasaji and McClure 1997).

Conclusion

Based on the field observation of *Adelges tsugae* attacks on the hemlock plant in every stages form seedlings to adult trees, assessment of predators in the field and evaluation of feeding potentials of the predator in laboratory following conclusions were made:

- The population of *A. tsugae* reach in peak by late spring or early summer. The infestation is not so severe to threat the existence of the host plant in the study area.
- The *Syrphus* sp. (syrphidae) and *C. septumpunctata* (coccinellidae) are the predators feeding on *A. tsugae* in Dhungharkha. Among these recorded predators, *C. septumpunctata* is an effective predator of hemlock woolly adelgid.
- The availability of hemlock woolly adelgids' predator depends upon the pest out break. The population of predator is positively correlated with the pest population.
- *Coccinella septumpunctata* is ploy phagous predatory beetle with consideralbe feeding efficiency on hemlock woolly adelgid so it could be the very important predator in keeping the population of adelgids in check.
- The male and female of *C. septumpunctata* have significantly different feeding potentials. The female are the more effective predators in terms of feeding capacity.

Recommendation

Relying on the findings of the study, the following recommendations have been made for the concerned authorities, researchers and other personnel particularly interested in the field:

- Though the pest number found below the economic injury level during the study period, any disbalance in natural predatory fauna may result in the outbreak of the pests and cause severe damage of the forest. So, proper conservation strategy should be made for the protection of hemlock forests from the attack of adelgids.
- More studies on the biology of hemlock woolly adelgids should be carried. The researchers are suggested to start their studies from early spring.
- The biological control of pest in forest setting is more stable than other methods. So further researches intented to find the potential biological control agents of hemlock woolly adelgid could be more worthy.
- Mass rearing and release of *C. septumpunctata* into the field to check the pest population could be valuable.
- Additional studies on oviposition, development and larval feeding efficiency on *Adelges* predatory flies, *Syrphus* sp. are suggested.
- Till date forest conservators and other concerning agencies have not realized the effects of *Adelges* in Nepal. Therefore, the concerned agencies are suggested to view the hemlock woolly adelgids as a pest of hemlock plant.

References

Brunetti, E. 1923. The Fauna of British India, Diptera (Pipunculidae, Syrphidae, Conopidae, Oestridae), **3**: 69-94.

Flower, J., Cohen, L. and Jarvis, P. 1998. Practical statistics for field biology (2nd ed). John Wiley & Sons, Chichester, England.

Kakkar, J., Rana, J.S. and Ombir. 2000. Feeding efficiency of seven spot ladybird *Coccinella septumpunctata* Linn. (Coleoptera: Coccinellidae) with cereal aphid, *Sitobion avenae*, Fab. (Homoptera: Aphididae) as prey. J Aphidol. **14**(1): 99-102.

Kapur, A.P. 1958. Coccinellidae of Nepal. Rec. Ind. Mus., Calcutta. **53**: 309-338.

McClure, M.S. 1990. Role of wind, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol **19**(1): 36-43.

McClure, M.S. 1987. Biology and control of hemlock woolly adelgid. Bull. Conn. Agric. Exp. Stn. 851.

McClure, M.S. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on Tsugae canadensis. Environ. Entomol. **20**(1): 258-264.

McClure, M.S. 1995. *Dipterobates humeralis* (Oribatida: Ceratozetidae): An effective control agent of hemlock woolly adelgid (Homoptera: Adelgidae) in Japan. Environ. Entomol. **24**(5): 1207-1215.

McClure, M.S. 1995. Managing hemlock woolly adelgid in ornamental landscape. Bull. Conn. Agric. Expt. Stn. 925

McClure, M.S. 2001. Biological Control of hemlock woolly adelgid in the eastern United States. FHTET-2000-08. Morgantown, WV: USDA Forest Service.

McClure, M.S., Salom, S.M. and Shields, K.S. 2001. Hemlock woolly adelgid. FHTET-2001-03. Morgantown, WV:USDA Forest Service.

Nagarkatti, S. and Ghani, M.A. 1972. Ecology and biology of predators. (vi) Coleoptera: Coccinellidae. In: V.P. Roa and M.A. Ghani (eds). Studies on predators of Adelges spp. in the Himalayas. Misc. publ. Commow. Inst. Biol. control **3**: 58-89.

Oldroyd, H. 1958. Collecting, preserving and studying insects. Hutchinson & Co. Ltd., London, England.

Rao, V.P. and Ghani, M.A. (eds). 1972. Studies on predators of Adelges spp. in the Himalayas. Misc. Publ. commow. Inst. Biol. Control 3.

Salom, S.M., Koke, L.T. and Zilahi-Balogh, G. 1999. Ecvalution of Laricobius nitrius as a potential canidate for biocontol of hemlock woolly adelgid. Hemlock woolly adelgid Newsletter, USDA Forest service.4.

Sasaji, H. and McClure, M.S. 1997. Description and distribution of *Pseudoscymnus tsugae* sp. nov. (Coleoptera: Coccinellideae), an important predator of hemlock woolly adelgid in Japan. Annu. Entomol. Soc. Am. **90**(5): 563-568.

Sharma, B.D. 1995. Gymnosperms morphology, systematics, reproductive biology. In: B.M. Johri (ed). Botany in India. Oxford & IBH Publishing Co. Pvt. Ltd., Delhi, India **2**:1-20.

Singh, R. and Malhotra, R.K. 1979. Bionomics of *Coccinella septumpunctata* Linn. Ind. J. Entomol. **14**: 244-299.

Young, R.F., Shield, K..S. and Berlygn, G.P. 1995. Hemlock wooly adelgid (Homoptera: Adelgidae): Stylet bundle insertion and feeding sites. Annu. Entomol. Soc. Am. **88**(6): 827-835.