ECTO AND ENDO PARASITES OF BATS IN KALLERI, GALCHHI RURAL MUNICIPALITY, DHADING, NEPAL

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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This is to recommend that the thesis entitled "**ECTO AND ENDO PARASITES OF BATS IN KALLERI, GALCHHI RURAL MUNICIPALITY, DHADING, NEPAL"** has been carried out by Ganesh Bahadur Shrestha for the partial fulfilment of Master's Degree of Science in Zoology with special paper Parasitology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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ABSTRACT

Bats are nocturnal mammals found everywhere except in the Antarctica and Arctic regions. They are important for the maintenance of the ecosystem in terms of pollination and seed dispersal, particularly in tropical regions as well as in controlling insect population that would otherwise pests. Bats have been reported as reservoir hosts for several pathogens including viruses such as Ebola, Nipah, Hendra and Lyssa. The purpose of the study was to determine the prevalence of ecto and endoparasites of bats (*Megaderma lyra* and *Eonycteris speleae*). Fourty four bats were captured using mist nets, at two different locations (Chamero Cave and Chamero Thok) in Kalleri, Galchhi Rural Municipality, Dhading, Nepal from 11 May 2017 to 14 June 2017. Ectoparasites were collected with the help of forceps and preserved in a vial containing 70% alcohol. Fecal sample were taken with the help of forceps and preserved in Potassium dichromate solution and examined by direct smear, flotation concentration and sedimentation concentration methods for examination of helminth parasites. The *Megaderma lyra* was found to harbor a three species of ectoparasites *Trichobius* sp*., Ischnopsyllus* sp. and *Spinturnix* sp. with predominant *Trichobius* sp. While the *Eonycteris speleae* was found to harbor five species of ectoparasites *Trichobius* sp*., Ischnopsyllus* sp*., Spinturnix* sp*., Basilia* sp*. and Ancystropus* sp. with predominant *Basilia* sp. From fecal examination, bat species of both study area were found to be infected with high prevalence of endoparasites. The *Megaderma lyra* was found to be infected with three species of endoparasites *Hymenolepis* sp., ascarid and hookwoom. While the *Eonycteris speleae* was found to be infected with four species of endoparasites *Hymenolepis* sp*.,* ascarid, hookworm and strongylid. The bats play important roles in the ecosystem and transmission of zoonotic diseases as they harbor vast range of parasites, further studies would be required to understand the bat parasite disease dynamics.

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LIST OF ABBREVATIONS

Abbreviated form	Details of abbreviations
LC	Least Concern
IUCN	International Union for Conservation of Nature
DD	Data Deficient
NiV	Nipah Virus
OIE	Office International des Epizooties
WAZA	World Association of Zoos and Aquaria
DPX	Distyrene, Plasticizer and Xylene
SMCRF	Small Mammal Conservation and Research Foundation
CDZ	Central Department of Zoology
ELSI	Ethical, Legal and Social Implications
GPS	Global Positioning System
WHO	World Health Organization

1. INTRODUCTION

1.1 Background Information

Bats are nocturnal mammals that are important for the maintenance of the ecosystem. Bats are the only flying mammals; they are found everywhere except in the Antarctica and Arctic regions (Airas, 2003) and perhaps are the most abundant, diverse and geographically dispersed vertebrates. Although bats are relatively common in temperate regions, they reach their greatest diversity in tropical forests (Hill and Smith, 1984; Vaughan *et al*., 2000). They are the second-most specious group of mammals after rodents. The most currently tally of mammals recognized 1117 species of bats worldwide which represent about one fifth (20%) of the 5418 known mammals species (Srinivasulu *et al.,* 2010). In Nepal, 53 species of bats are reported which represents about 5% of the world bat diversity (Thapa, 2010).

1.2 Bat Biology

Bats are often divided into two major groups, usually gives the rank of suborders-Megachiroptera and Microchiroptera. Although these groups probably do not represent monophyletic lineages, these are several relevant ecological differences between them. Megachiroptera includes one family (Pteropodidae) and about 186 species (Srinivasulu *et al.,* 2010). All feed primarily on plant materials, either fruit/nectar or pollen and they have a good sense of smell which they use to locate ripe fruits during their hunting at night (Thapa, 2010). They also rely on vision to orient in the dark at night and thus have large prominent eyes and most species of fruit bats can't echolocate and roost mainly in trees and shrubs, except the Egyptian fruit bat (*Rousettus aegyptiacus*) which are able to find their way by echolocation (Rosevear, 1965).

The remaining 16 families (around 930 species) belong to Microchiroptera (Wilson and Reeder, 2005). Microchiropteran families prey upon insects to some extent, a minority have evolved to feed on vertebrates, blood, fruit, nectar and pollen, (Altringham, 1996). Microchiropterans have the ability to use echolocation during hunting and to avoid obstacle. Microchiropterans are either tree hollow roosting or cave roosting bats and they inhabit a wide range of habitats with vegetation cover ranging from wet and dry forests, swamps, rain forests to open farm lands and suburban areas (Rosevear, 1965). Microchiropterans, unlike megachiropterans, have labile body temperatures and some hibernate (Hill and Smith, 1984; Vaughan *et al*., 2000).

1.2.1 *Eonycteris spelaea* (Dobson, 1871)

Eonycteris spelaea is commonly known as Dawn Bat and also called Mirmire Chamero in Nepali. It is Least Concern as International Union for Conservation of Nature Status and Data Deficient in National status (Thapa, 2010). It belongs to family Pteropodidae. It is medium sized fruit bat resembling to *Rousettus* but differes characteristically in absence of claw on second digit and muzzle also comparatively elongated while tail is short and its tip protrudes from the interfemoral membrane (Thapa, 2010). Large kidney shaped glands are present on either side of the anal opening and dorsal pelage is dark brown, relatively paler on the back of the head and shoulders while ventral pelage is mottled grey-brown which feeds upon nectar of chiuri and disperse their seeds and it is also believed as the long

distance forager that enhances to expand the genetic dispersion of bat pollinating plants (Thapa, 2010).

1.2.2 *Megaderma lyra* (Geoffroy, 1810)

Megaderma lyra is commonly known as Greater False Vampire Bat and also called Nakkali Boksi Chamero in Nepali. It is LC in both IUCN and National Status (Thapa, 2010). It is insectivorous bat belonging to family Megadermatidae. Pinnae are characteristic and oval shaped, large with fringe of white hairs on inner margins which are joined at half to one-third of their length and tragus is bifid; its posterior process is taller relatively, forehead and upper cheeks of the face is hairy while snout is naked and flesh colored, noseleaf is erect, straight and about 10 mm in height; which has a longitudinal ridge and a simple rounded horizontal base, pelage is fine, soft and moderately long, dorsal pelage is mouse grey with slightly brown shades, ventral pelage paler with hair tips of throat and belly white while hair bases grey to dark grey and wings are attached to the base of the outer toe (Thapa, 2010). It inhabits the dry as well as humid areas with agriculture fields and wetlands and it roosts solitarily and in small to large colonies ranging upto several hundred individuals, it has been found roosting in caves, old buildings, thatched huts etc and it flies silently and maneuvers close to the ground which feed upon varieties of insects; however vary seasonally, also vertebrates (generally frogs, reptiles and rodents) (Thapa, 2010).

1.3 Bat Diseases

Bats have been implicated in the emergence of many high profile zoonotic diseases of public health importance including rabies, Ebola, Nipah, Hendra and Severe Acute Respiratory Syndrome (SARS) related coronaviruses (Calisher *et al.,* 2006). Their relevance to human health has increased the interest in bats as potential reservoir hosts and vectors of zoonotic pathogens (Muhldorfer, 2013). The high mobility of bats, broad distribution social behavior (communal roosting, fission-fusion social structure) and longevity also make them ideal reservoir hosts and sources of infection for various etiologic agents (Bai *et al.,* 2011).

Bats harbour deadly viruses, bacteria, protozoans and other disease-causing pathogens. For example Nipah virus (NiV), a deadly virus harboured by bats, caused significant human mortality (~40%) during its initial outbreak in Malaysia in 1998 (Chua *et al*., 2000) and has emerged repeatedly in Bangladesh and India since 2001 (Lo *et al*., 2012; WHO, 2013). Hendra Virus Diseases is rare, it could cause fatal respiratory or neurological disease in humans. Marburg virus disease is another rare hemorrhagic disease that affects humans and other primates causing serious illness with high mortality. The reservoir host of this virus is the African fruit bat, *Rousettus aegyptiacus.* However, they do not show any obvious signs of illness (Swanepoel *et al.*, 2007).

1.4 Parasites

Parasites are any organisms that live in or on hosts, deriving benefit at expenses of host. Parasites are capable to change host population size and may affect hosts fecundity, morbidity and mortality or indirectly hosts immunity affecting its physical condition (Hechinger and Lafferty, 2005; Nichols and Gomez, 2011). Wildlife themselves are the source of infectious diseases and have infection to peripheral animals (OIE, 2000; Lisle *et al*., 2002; WAZA, 2005; Ahasan and Azam, 2007). Like all other animals, bats are associated with internal and external parasites (Hill and Smith, 1984).

1.4.1 Ectoparasites

Ectoparasites are organisms which inhabit the skin or outgrowths of the skin of another organism (the host) for various periods, and may be detrimental to the host (Hopla *et al*., 1994). They live in the fur, wing membranes and other parts of the host's body where they feed on host's blood. Most of them have flattened bodies for easy movement through the fur of their hosts and sucking mouth parts for sucking blood from their hosts. Bats harbour an unusual number of ectoparasites as compared with many other mammals (Lewis, 1983) with a number of them being specific. Bat flies, for example, spend most of their life on their hosts, only leaving their hosts as larvae and so follow their hosts' geographical distribution closely (Marshall, 1982; Dick and Gettinger, 2005). The family of flies (Streblidae) is extremely specialized such that some species have lost their ability to fly during evolution and live now as 'bat lice' in the fur of bats (Wund and Myers, 2005). Mites are similarly host-dependent and complete their entire life-cycle on the body of their host (Christe *et al*., 2000). For example family Spinturnicidae are host specific and only occur on bats.

Bats commonly harbor external arthropod parasites such as flies, mites, ticks, bugs and fleas. The arthropod ectoparasites of bats belong to the Siphonaptera, Diptera, Hemiptera, Dermaptera and Acarina (ticks and mites) but they are not necessarily restricted to bats (Whitaker, 1988). 687 bat ectoparasites insect species are known, belonging to the Dermaptera, Hemiptera, Diptera and Siphonaptera orders and seven families. Six among seven families are exclusively dependent on bats (Marshall, 1982). The parasite species that infest bats exhibit a range of host specificity, some are found on one or a few species; while some occur on a wide variety of bat species (Hill and Smith, 1984).

1.4.2 Endoparasites

Endoparasites are parasites that live in the internal organs or tissues of its host. Bats are known to harbor several protozoans that cause malaria (eg. *Plasmodium, Hepatocystis, Nycteria* and *Polychromophilus*) (Schaer *et al*., 2013) as well as intestinal parasites. Helminth and protistian (protozoan) parasites that are known to occur in bats are both morphologically and phyllogenetically diverse and include many species of tapeworms (cestodes), flukes (trematodes), nemata (nematodes), eimerians (coccidia), tongue-worms (pentastomes) and flagellates (trypanosomes) and many flatworms (Cestoda and Trematoda) *Posthodendrium panouterus* and *Hymenolepis kerivoulae* and roundworms (Nematoda) *Rictularia charaphani* and *Histostrongylus coronatus* (Okafor *et al*., 2004) spend at least part of their life cycle within the tissues of bat hosts (Wund and Myers, 2005). It was not until 2002 that the partial life cycle was documented for one of the most common nematode parasites of bats in the new world, a filarioid nematode of the genus Litomosoides (Bain *et al*., 2002).

1.5 Objectives

1.5.1 General objective

To determine the prevalence of ecto and endoparasites of bats (*Megaderma lyra* and *Eonycteris speleae*) in Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

1.5.2 Specific objectives

- To identify the taxonomic details of ectoparasites of bats.
- To determine the prevalence and intensity of endoparasites of bats.

1.6 Justification of the study

Bats are important in pollination and seed dispersal. Many rainforest trees depend on bats for pollination and seed dispersal which is particularly important in facilitating regrowth after forest clearance (Knowles, 2009). Similarly, many tropical plants depend on the activities of bats for the distribution of their seeds. It is estimated that there are some 300 bat-dependent valuable plant products, including chewing gum, tequila, sisal, medicines, dyes and fuel (Knowles, 2009). Guano which is bat droppings is used as fertilizer. Some bats are keystone species on which the health of whole ecosystems depend. Insectivorous bats significantly reduce insect populations that would otherwise be pests. There is therefore the need to conserve these animals as they are the major species on which whole ecosystems depend on for their sustainability.

Nepal has very less information about bats and their parasites. The study about bats is important as they are the indicator of healthy ecosystem and also considered as tool as they are effective on biological control of insects, pollinations for plants, seed spreading, controlling crop parasites and help to keep healthy environment. Since the bat diseases including parasitic information in Nepal is lacking, this study will provide the baseline information about ecto and endoparasites of bat.

2 LITERATURE REVIEW

2.1 Ectoparasites of bats

Bats harbour an unusual number of ectoparasites as compared with many other mammals (Lewis, 1983). Bat flies, for example, spend most of their life on their hosts, only leaving their hosts as larvae and so follow their hosts' geographical distribution closely (Marshall, 1982; Dick and Gettinger, 2005). The family of flies (Streblidae) is extremely specialized such that some species have lost their ability to fly during evolution and live now as 'bat lice' in the fur of bats (Wund and Myers, 2005). Mites are similarly host-dependent and complete their entire life-cycle on the body of their host (Christe *et al*., 2000). For example family Spinturnicidae are host specific and only occur on bats.

Ectoparasite infestation is common among all the warm blooded animals. The ectoparasites of bat from African countries were reviewed. Obame-Nkoghe *et al. (*2016) reported 439 bats infested by batflies in Gabon, out of 1,154 bats. Similarly, Fain *et al.* (1984) found new subspecies differs from the typical form found on a Molossid bat in Africa and from the subspecies longispina. Maa (1971) recorded 16 streblid species of the megachiroptera and five species were re-assigned to a new genus and only one was retained in the genus *Brachytarsina* (Nyteribosca). Nartey (2015) found four species of bat flies and *Nycteribia alternate* and *Cyclopodia greefi greefi* were the most common bat flies recorded in Ghana. Wood (2012) reported four species of flies in *Miniopterus natalensis* in South Africa.

Lukoschus *et al.* (1973) found one new species (*Psorergatoides glossophagae)* of mite in Surinam bats in New Guinea. Similarly, Nartey (2015) reported eithteen species of mites and the most abundant ectoparasite group found on bats were mites; where the species most frequently encountered were *Spinturnix Americana, Carpoglyphus sp* and *Ancystropus zeleborii* in Ghana. Wood (2012) reported eight species of mites, one flea and one tick in *Miniopterus natalensis* in South Africa.

The ectoparasites of bats reports from various countries of American continents were reviewed. Imaz *et al.* (1999) found 664 ectoparasites from 160 potential host in Biscay (N lberian peninsula) and Hofstede *et al.* (2004) collected 773 ectoparasitic bat flies on 455 bats from the Lamanai area of Belize. Similarly, Graciolli and Azevedo (2011) found ectoparasites of bats, with a description of a new species of Synthesiostrebla Townsend (Diptera, Streblidae) from Brazil. Moras *et al.* (2013) found 14 bat fly species from bats in Southern Minas Gerais, Brasil. More than 36500 bat flies of 116 species were captured from more than 25000 bats representing 130 species in Venezuela (Dick and Patterson, 2008). Fritz (1983) found female bats were significantly higher numbers of bats flies in Hawaii, USA and *Trichobius joblingi*, *Speiseria ambigua* and *Strebla guajiro* were found on *Carollia.* Gordon and Ross (2010) surveyed 20000 bats of various species for the parasites in Arizona and found Streblidae as major parasites. Dick *et al.* (2003) found 13.2% ectoparasitic arthropods of four species of the host individuals at Pennsylvania's Canoe Creek State Park. A total of 1,259 specimens from six species in the Streblidae family were collected from 332 bats in caves within the Cafuringa Environmental Protection Area in the Federal District of Brazil (Souza and Antonini, 2011). The sixteen species of streblid bat flies were collected from 10 bat species of the family Phyllostomidae

where thirteen of these streblid species were recorded for the first time in Pernambucu, Brazil (Soares *et al.,* 2008). Dick and Gettinger (2005) collected 2,467 bat flies representing 11 genera and 31 nominal species of Streblidae, of which 6 genera and 24 species were new records for Paraguay. Patterson *et al.* (2008) examined the correlations between prevalence (proportion of infested individuals), mean intensity (number of parasites per infested individual) and the number of bat fly species parasitizing bats in Venezuela. Of 181 bats belonging to 16 species, 10 (34.1%) were infested by streblid flies in southeastern Brazil (Almeida *et al.,* 2011). Krichbaum *et al.* (2009) found ectoparasites as bat flies of a previously undescribed species of the *Nycterophilia* genus from all three species of bats *Mormoops blainvillei, Pteronotus quadridens* and *Monophyllus redmani* from Puerto Rico. Autino *et al.* (2011) reported four species of ectoparasites *Neotrichobius ectophyllae, Strebla galindoi, Strebla paramiralis* and *Myodopsylla wolffsohni wolffsohni* of bats in Peru.

Moras *et al.* (2013) found 9 mite species from bats in Southern Minas Gerais, Brasil. Similarly, Silva and Graciolli (2013) found nine genera and 11 species of bats were parasitized with *Periglischrus torrealbai, Periglischrus tonatii* and *Periglischrus iheringi* from Pantanal, Brasil and *Periglischrus iheringi* was the most abundant mite species. Yunker (1958) examined for parasitic mites in eastern United States and found Spinturnicidae, Dermanyssidae and Trombiculidae. Almeida *et al.* (2011) found bats were infested with 33.5% by spinturnicid mites and 8.3% by macronyssid mites in southeastern Brazil. Foster and Mertins (1996) reported eight species of mites with *Chiroptonyssus robustipes* being most prevalent (100%) and the mites *Ewingana longa, Dentocarpus macrotrichus* and *Notoedres (Bakeracarus) sp.* were reported for the first time infesting bats from Florida. Guzman-Cornejo *et al.* (2003) reported 12 mite from the states of Puebla, Zacatecas and Durango.

The ectoparasites of bats from Asian continents had very few works as compared to other continents. Hassan *et al.* (2010) reported the ectoparasites of lesser mouse eared bat, *Myotis blythii* inside the Mathi-Dasth cave in Kermanshah province, Iran. Similarly, Aroon *et al.* (2015) found 31.82% of bats were infested with ectoparasites belonging to 4 families, 7 genera and 7 species in northeastern Thailand. Alvarez *et al.* (2016) reported five species of Streblidae from 11 bat species in Naujan Lake National Park, Mindoro Oriental Proviece, Philippines. Two hundred twenty five ectoparasites representing 36 species and seven families were collected from 140 bats captured in mount makiling, Laguna, Philippiness (Alvarez, 2013).

Uchikawa and Kobayashi (1978) found 12 species of fur-mites belonging to the family Myobiidae and 16 larvae of Argasidae in Thailand. Similarly, Wilson and Wodzicki (1977) reported the mite *Meristaspis calcarata* from *Pteropus t. tonganus* Quay and Gaimard in the Pacific region. Delfinado and Baker (1963) reported four genera in Spinturnicidae from bats collected in the Philippines. Lukoschus *et al.* (1973) found one new species (*Psorergatoides glossophagae)* of mite in Surinam bats in Burma.

Alvarez *et al.* (2016) reported seven species of Nycteribiidae and one species of Ischnopsyllidae (Siphonaptera) from 11 bat species in Naujan Lake National Park, Mindoro Oriental Proviece, Philippines.

Bat ectoparasites reports from Europe have shown maximum studies on mites than other ectoparasites. Ferenc and Myslajek (2003) discovered 8 species of mites (including one with two subspecies) in Polland. Orlova and Zapart (2012) discussed the species composition and relationship between ectoparasites of pond bats species in mixed colonies in northern Polland. Similarly, Kristofik and Danko (2012) found 14 species of the family Spinturnicidae with two subspecies belonging to three genera: *Paraperiglischrus, Eyndhovenia* and *Spinturnix* in Slovakia of which six fauna were new recorded. Jaunbauere *et al.* (2008) found *Spinturnix vespertilionis* and *Nycteribia pedicularia* on sixteen species of bats in Latvia. Valdez *et al.* (2009) recorded 10 families and 13 genera of insect or acari as the major species mites *Macronyssus crosbyi, Alabidocarpus calcaratus, Acanthophthirius lucifugus* and *Alabidocarpus nr. Eptesicus* in 634 fresh hosts and 1524 museum specimens of bats from Mexico and southern Colorado. Lukoschus *et al.* (1973) found one new species (*Psorergatoides glossophagae)* of mite in Surinam bats in Venezuela. Orlova (2011) reported twenty species belonging to 8 families of insects and mites from six bat species in northern Eurasia.

Rupp *et al.* (2004) reported 33 species of eight parasitic families of fleas (Ischnopsyllidae), batflies (Nycteribiidae), bugs (Cimicidae), mites (Spinturnicidae, Macronyssidae, Trombiculidae, Sarcoptidae) and ticks (Argasidae, Ixodidae) in Bavaria, Germany.

In Nepal, very few work has been carried out on ectoparasites of bats. Dahal and Thapa (2010) had reported each specimen of Cimicidae and Ischnopsyllidae, two specimens of Spinturnicidae and fifteen specimens each of Nycteribiidae and Streblidae ectoparasites associated with bats from Kathmandu Valley. Similarly, Mitchell and Punzo (1976) described 56 individuals of 11 species of bats of Nepal consisted of 93 ectoparasites of nycteribiid flies, argasid ticks, laelapid, spinturnicid, mites and several ischnopsyllid fleas.

2.2 Endoparasites of bats

Bats also serve as host for a number of endoparasites including protozoans (*Plasmodium* and *Trypanosoma* species) (Schaer *et al.,* 2013) and platyhelminthes (cestodes, nematodes and trematodes) (Okafor *et al.,* 2004), but usually show no symptoms to the high burdens of endoparasites and pathogens that they harbour and transmit (Wenzel *et al.*, 1996).

The endoparasites reported in bats include protozoans (*Plasmodium* sp., *Nycteria* sp., *Hepatocystis* sp. and *Polychromophilus* sp.) (Schaer *et al*., 2013), trypanosomes (Thomas *et al.*, 2007) and helminths (trematode s, cestodes and nematodes) (Okafor *et al.,* 2004; Nogueira *et al.,* 2004). Endoparasites are very evasive parasites which find various ways of evading the immune system of their hosts. They lodge in organs such as the heart, liver and lungs of their host and also in fluids such as the blood and lymph and in fact every part of its host. The well adapted nature of endoparasites can be seen in the adult *Toxocara pteropodis* which are found only in the intestines of suckling young flying fox which pass the eggs in faeces (Nelson, 1989).The adult bats get infected by ingesting faeces from foliage in the roosting habitats. The eggs of these helminths hatch and move to the liver of adult male bats and mammary glands of the adult female bats.

The endoparasites of bats from African continents were reviewed. Schaer *et al. (*2015) surveyed insectivorous bats from tropical Africa, including new isolates of species of the haemosporidian genus *Nycteria*. Similarly, Gay *et al. (*2014) reported that bats were found

infected with Nycteria parasites by (22%) prevalence in South Sudan. Wood (2012) reported 11 helminth (one cestode, seven nematode and three trematode) species in *Miniopterus natalensis* in South Africa.

The endoparasites of bats reports from American continents were higher than other continents. Nogueira1 *et al. (*2004) found endoparasites from a sample of 50 stenodermatine bats collected mainly over lick sites at the Parque Nacional da Serra do Divisor, westernmost extremity of Amazonian Brazil. Similarly, Pistole (1988) found sixty three percent of the bats were infected with 1 or more species of parasite with six nematodes species, 4 cestode species and 20 digenean species were recovered and *Paralecithodendrium transversum* and *Ochoterenatrema diminutum* were most commonly occurring species being found in high intensities or prevalence in the most bat species in Terre Haute, Indiana. Hilton and Best (2000) reported the bats were infected with 37.3% helminthes, 4.3% cestodes, 14.5% nematodes and 23.8% trematodes and 4.6% were parasite by two types of helminths and one had all three types of helminthes in Alabama. Oviedo *et al.* (2012) reported *Biacantha normaliae* new species of nematodes in common vampire bat from the Yungas, Argentina. Mcallister *et al.* (2007) found the bats were infected with lecithodendrid trematodes, *Acanthatrium alicatai* and nematodes (*Seuratum cancellatum)* and acoccidian (*Eimeria antrozon)* in Southwestern Texas. De Albuquerque *et al.* (2016) found 20.89% parasites of total bats studied representing *Anenterotrema eduardocaballeroi, Anenterotrema liliputianum, Ochoterenatrema caballeroi, Tricholeiperia sp., Parahistrostrongylus octacanthus, Litomosoides guiterasi, Litomosoides brasiliensis, Capillariinase* sp. and *Hymenolepididae* sp. in Amazonia. Cardia *et al.* (2015) found a new species nematode, *Pterygodermatites (Paucipectines) andyraicola* sp. from the small intestine of 4 neotropical species of Molossidae bats captured in Sao Paulo State, Brazil. Mcallister (2008) found two plagorchiid trematodes in small intestine of *Myotis ciliolabrum* in south Dakota. Both trematodes were identified as *Plagiorchis microcanthos* (Macy, 1931).

Heddergott and Steinbach (2015) reported new records of the trematode, *Plagiorchis microcanthos* from three North American bats. Mcallister *et al.* (2004) found twelve lecithodendrid trematodes, *Prosthodendrium transversum* 20% and seven ascarid nematodes, *Seuratum cancellatum* 20% were found in small intestine of *Myotis septentrionalis* and ova of nematode *Capillaria palmate* 22% in the feces of *Eptesicus fuscus* from Pipistrelle Mine, Polk County, Arkansas. Williams (1957) reported total of twelve species and one subspecies of helminths, including two new species representing one cestode (*Hymenolepis* roudabushi), nine trematodes (*Prosthodendrium transversum, P. ascidia navicula, Acanthatrium microcanthum, A. eptesici, A. lunatum* sp., *A. obovatum* sp., *Acanthatrium.* sp., *Limatulum gastroides* and *Plagiorchis micracanthos*) and three species of nematoda (*Physocephalus sexalatu, Ascarops strongylina* and *Capillaria sp.*) from bats in United States. Lotz and Font (1983) found fifteen species of Lecithodendriidae from *Eptesicus fuscus* in Wisconsin and Minnesota: *Acanthatrium eptesici, A. microcanthum, A. oligacanthum, A. pipistrelli, Allassogonoporus marginalis, Glyptoporus noctophilus, Ochoterenatrema breckenridgei, O. diminutum, O. travassosi, Paralecithodendrium chilostomum, P. macnabi, P. naviculum, P. nokomis, P. swansoni* and *P. transversum.*

Foster and Mertins (1996) found eleven species of helminthes parasites which included 7 trematodes, 3 nematodes and 1 cestode, all of which were new records for bats from Florida where *Vampirolepis decipiens* and *Ochoterenatrema breckenridgei* was first record for *Tadarida brasiliensis* in the United States and the nematodes *Molinostrongylus delicatus* was the most prevalent helminth collected (64%). Krichbaum *et al.* (2009) found cestode *Vampirolepis christensoni* and nematode *Capillaria pusilla* from Puerto Rico. Guzman-Cornejo *et al.* (2003) examined ninety-eight Mexican free-tailed bats, *Tadarida brasiliensis mexicana* and found 5 helminth taxa (3 digenean, 1 cestode and 1 nematode) from the states of Puebla, Zacatecas and Durango.

The very few work had done in Asian continents. Sawada (1970) examined three species of common Japanese cave-bats, *Rinolophus ferrumequinum, R. cornutus* and *Miniopterus schreibersi* in Okayama Prefecture for the presence of cestodes and found eleven of these twenty-four bats harbored five species of hymenolepid cestodes, of which were new ones and the cestodes were identified as *Insectivorolepis okamotoi* sp., *Vampirolepis isensis, Rodentolepis mocrotesticulatus* sp. and *Insectivorolepis niimiensis* sp. Matskasi (1973) examined 278 bats specimens representing 36 species collected in India, 22 trematode species were identified and three new species (*Neoheterophyes topali, Prosthodendrium robustum* and *Parabascus ghati*) were described.

The endoparasites of bats reports from European continents were reviewed here. Afonso *et al. (*2016) reported the prevalence for the parasite *Eimeria hessei* ranged from 0% to 80% among colonies of twenty lesser horseshoe bat in Western and Central Europe. Similarly, Horvat *et al.* (2015) found fifteen individuals (88.2%) were found to be infected with helminth parasites and five parasites species were identified: three digeneans (*Plagiorchis sp., Prosthodendrim longiforme, Lecithodendrium linstowi*) and two nematodes (*Strongylacantha glycirrhiza, Litomosa ottavianii*) in bats from Serbia. Esteban *et al.* (2001) found five species of trematodes, *Plagiorchis* (*Plagiouchis*) sp., *Lecithodendrium* (*Lecithodendrium*) *linstowi, Prosthodendrium* (*Prosthodendrium*) sp., *Pycnoporus heteroporus* and *Parabascus semisquasus* and species of cestode, *Hymenolepis pipistrelli* in bats from Spain. Lord *et al.* (2012) found sixty-eight of 90 bats (76%) were infected with at least 1 species of helminth and all helminthes were digenean trematodes and the following species were identified in *Pipistrellus pipistrellus* (51%), *Lecithodendrium linstowi* (80.4%), *L. spathulatum* (19.6%), *Prosthodendrium sp.* (35.3%), *Plagiorchis koreanus* (29.4%) and *Pycnoporus heteroporus* (9.8%) in England. Saoud and Ramadan (1976) found 43.5% bats were infected with one or more species of helminth parasites, Of the 555 infected bats 435 harbored one group only of parasites, either trematodes, cestodes or nematodes and in the majority of cases it was trematode infection and double infections with two of the three groups of parasites were found in 118 bats while triple infections with three groups were encountered in only two bats from different localities in Egypt.

3 MATERIALS AND METHODS

3.1 Study area

Dhading (27'40" E to 28'17" E and 80'17" N to 84'35" N) is the only district of Nepal which ranges from the mountain Ganesh Himal to the Churevawar Pradesh of Terai (Chitwan). The transnational Prithvi Highway connecting Kathmandu and Pokhara runs through the southern portion of the district making for easy access to the Kathmandu valley. The roads parallels the Trishuli River. The district is bounded by Kathmandu, Rasuwa and Nuwakot from east, Gorkha from west, Rasuwa and Tibet from north and Makwanpur and Chitwan from south.

The climate of Dhading is characterized by temperate climate and influenced by the tropical monsoon. Dhading is 80% farmland and 20% forest. The surrounding hilly area is covered with forest of *Alnus nepalensis, Pinus roxburghii, Terminalia alata, Shorea robusta, Dalbergia sissoo, Acacia catechu, Prunus sps,* bamboo and chiuri as major vegetation. The forest consists of hills and many caves. Caves are the best resident for nocturnal animals like bats.

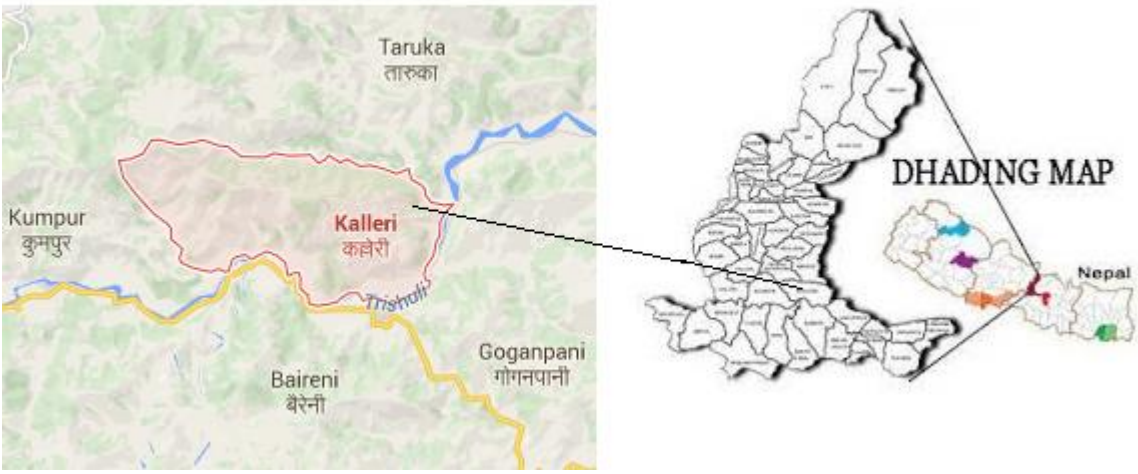

Figure 1: Kalleri, Galchhi Rural Municipality, Dhading, Nepal.
Source: http://www.todaykhabar.com/2016/07/03/53595/dhading-district-map/

3.2 Materials

Gloves**,** mist net**,** bamboo sticks**,** sterile vials**,** cool box**,** refrigerator**,** beaker**,** glass rod**,** measuring cylinder**,** tea strainer**,** centrifuge tube**,** centrifuge machine**,** dropper**,** slides**,** cover slips**,** needle**,** cotton**,** stage micrometer**,** ocular micrometer and electric microscope.

Chemicals

Potassium dichromate, distilled water, saturated Sodium Chloride solution, methylene blue, Lugol's Iodine solution, alcohol series, xylen and DPX.

3.3 Methods

3.3.1 Bat capture

Bats were captured in 6m by 9m mist nets at the entrance of caves, stone crevices and near the souce of water at the forest area. The nets were set from 6 pm to 9 pm and inspected every 10 minutes to ensure that bats captured did not stay too long in the net struggling. Bats hunt at night for food and therefore it was necessary to work on them quickly and release them to go and feed. Captured bats (Figure 2) were removed and placed in aerated sacs and brought to the working area for examination for parasites. Thick gloves were worn while removing the bats from the nets to prevent scratches and bites.

Bats were identified with the help of a field guide 'Bats of Nepal' by Small Mammals Conservation and Research Foundation (SMCRF) then inspected for parasites.

Figure 2: Bats entangled in a mist net.

3.3.2 Collection of ectoparasites

Individual bats were carefully handled and examined for ectoparasites (Figure 3 and Figure 4). The fur, wing membranes and ears of each bat were carefully searched with a light and visible ectoparasites were carefully picked with the forceps and placed in vial with half filled with 70% alcohol. Placing the ectoparasites in 70% alcohol ensured that the specimens were not dehydrated and that important features remained intact. Each sample was then labelled with a unique number and locality. This information as well as other data including the species of bat were recorded on a data sheet for future reference.

Figure 3*:* Handling *Megaderma lyra* and *Eonycteris speleae*

Figure 4: Ectoparasites collection

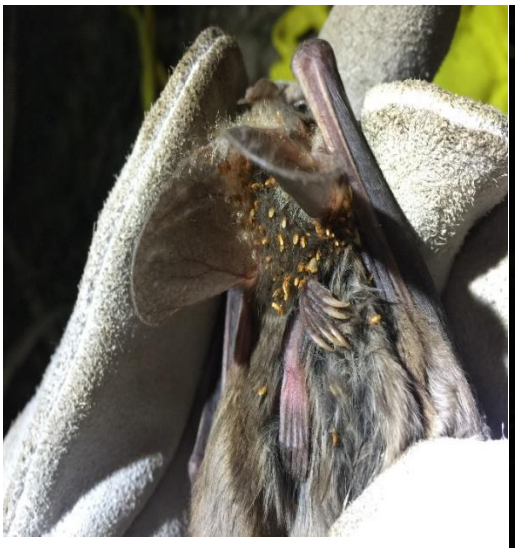

Figure 5: Ectoparasites on bat

Figure 6: Bat hanging on Bamboo

Figure 7: Flying bat inside the cave

Figure 8: Bats hanging inside the cave

3.3.3 Preparation of slides

The permanent slides of collected samples were prepared at the laboratory of Central Department of Zoology, Tribhuvan University. The ectoparasites were boiled in Potassium hydroxide for about one hour and then subjected to alcohol series of 30%, 50%, 70%, 90% and 100%. The specimens were mounted upon glycerine and finally the specimens were mounted on DPX and covered with cover slip. These slides then were observed under the electric microscope at 10x40x zoom and microphotography were taken at CDZ lab showing all taxonomic details.

3.3.4 Identification of ectoparasites

Ectoparasites were identified with various identification keys (Maa, 1962; Delfinado and Baker, 1963; Hopla *et al.,* 1994; Whitaker *et al.*, 2007) for mites, fleas, louse and flies.

3.3.5 Sampling of fecal pellets/Preservation of endoparasites of bats

Fecal pellets of the bats were taken from the floor of caves and also from the gloves for examination of helminths parasites. During the sampling process, fecal pellets passed out by the bats were collected with the help of forceps and stored in a vial with half filled 2.5% potassium dichromate. This was to preserve and maintain the integrity of any eggs or cysts that the fecal pellets may contain.

Figure 9: Searching fecal matter

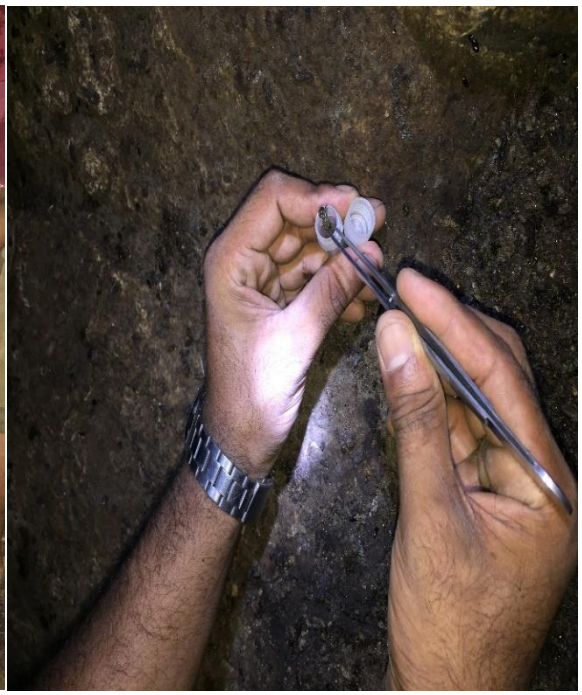

Figure 10: Collection of fecal matter

3.3.6 Lab process

Fecal samples were processed for microscopic examination of ovum/oocysts/cysts/larvae and were identified according to the morphology and quantitative estimation by using direct method and concentration method (flotation and sedimentation).

3.3.6.1 Preparation of normal saline

Normal Saline was prepared by dissolving 8.5 gm of sodium chloride in 1000 ml of distilled water which was used in unstained preparation (Zajac and Conboy, 2012).

3.3.6.2 Preparation of Iodine solution

The iodine solution was prepared by dissolving 10gm potassium in 100ml of distilled water and 5gm of iodine crystals (powdered) were slowly added in it. The solution was then filtered and kept in a stopper. It was diluted about 5 times distilled water. The solution was used in a stained preparation for the visualization of internal organelle of the protozoan parasites as well as helminth eggs (Zajac and Conboy, 2012).

3.3.6.3 Unstained smear preparation of fecal matter

A small portion of fecal sample was taken with the help of small stick and emulsified with freshly prepared normal saline on a clean glass slide. A clean cover slip was placed it and excess of fluid was removed with the help of cotton.

3.3.6.4 Stained preparation of fecal smear

The stained smear preparation was required for the identification and study for nuclear membrane of the heminths eggs. The stained smear was prepared in the similar manner as prepared to unstained smear.

3.3.6.5 Methods of observation

Both stained and unstained smear preparations were first observed under low power (10X objective) of the compound microscope. Examination was started from one end of the slide to another for whole field examination. Samples were examined under high power (40X) when necessary for detailed diagnosis.

3.3.6.6 Flotation concentration and sedimentation concentration Method (Dryden *et al.*, 2005)

3.3.6.6.1 Flotation method

Approximately 3 gm of fecal sample were taken in a beaker and 20 ml of water were added. Then the sample were mixed or grinded with the help of mortar or pestle and filtered the solution by tea strainer. The filtrate solution were poured into a centrifuge tube of 15 ml and centrifuged at 2,000 rpm for 5 minutes. The tube's water were replaced with saturated sodium chloride solution and again centrifuged. After centrifuge more saturated sodium chloride solution were added to develop convex surface at the top of the tube and one drop of methylene blue (to stain) where a cover slip were placed at top for a few minutes and then cover slip wer removed and placed on a slide and examined at 10X and 40X. Photographs of eggs of helminths were taken and identified based on egg's color, shape and size and with the help of different keys.

3.3.6.6.2 Sedimentation method

Saturated salt solution were removed gently from the test tube after examination of floated portion and poured the sediment content into watch glass and stirred the content gently to mix it. One drop from the mixture were taken to prepare a second slide. The specimen were stained with iodine wet mounts solution.

In this way, two slides were prepared from one sample and examined under 10X and 40X magnification of microscope to detect eggs of helminthes parasites.

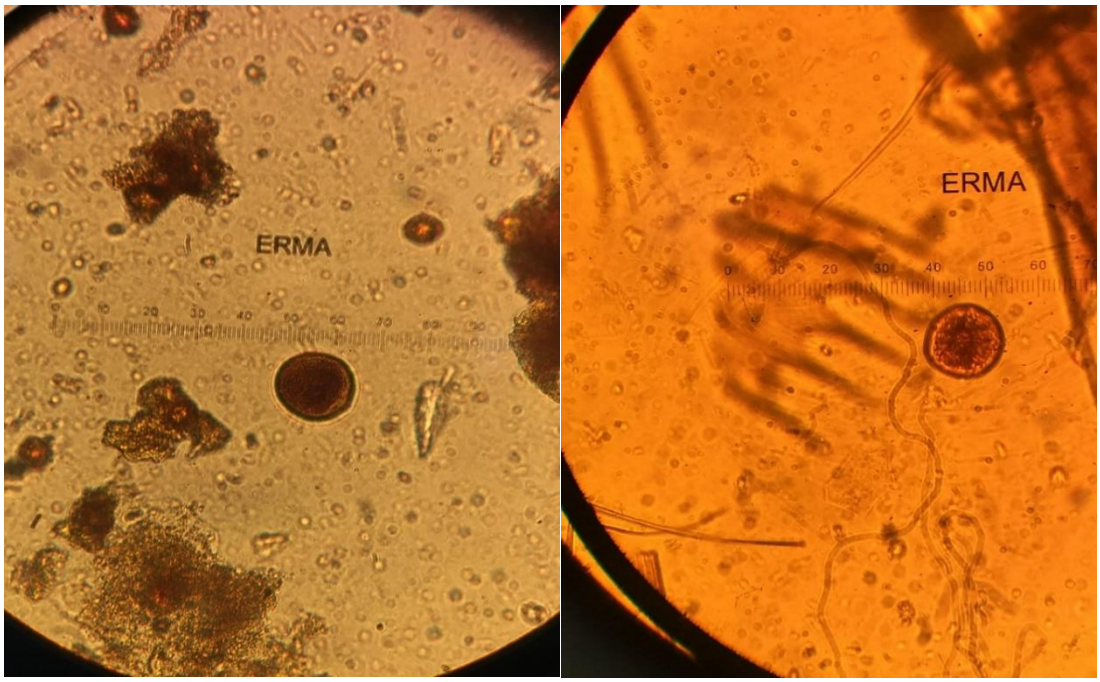

Figure 11: Ascarid eggs (31.2 µm)

Figure 12: Ascarid (84 µm)

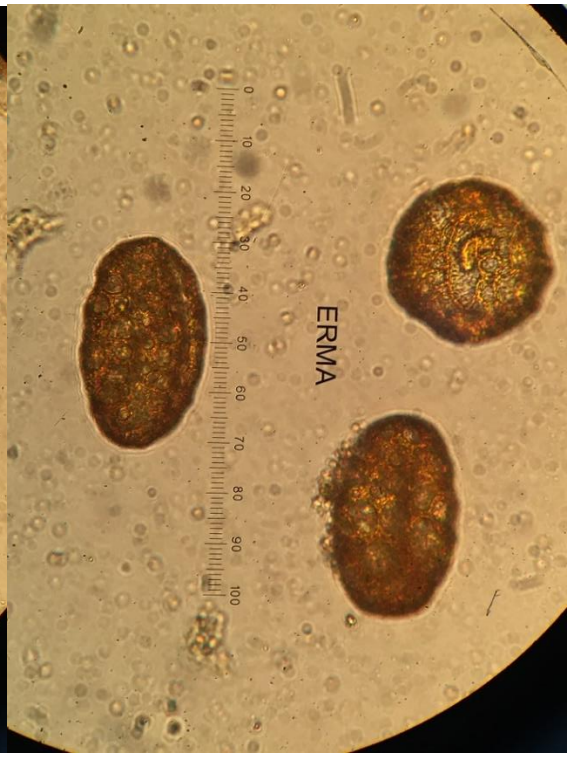

Figure 13: Hookworm (172.8 µm x 86.4 µm)

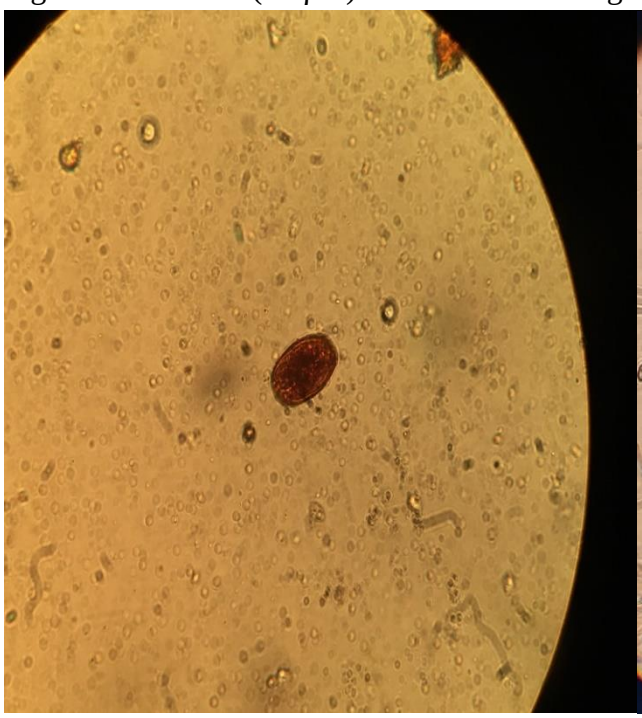

Figure 14: *Hymenolepis* sp.

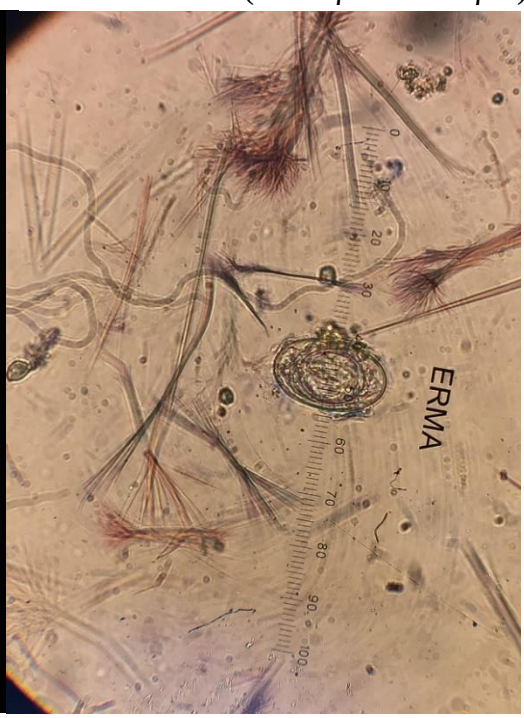

Figure 15: Strongylid (52.8 µm x 39.2 µm)

Figure 16: Preservation of samples

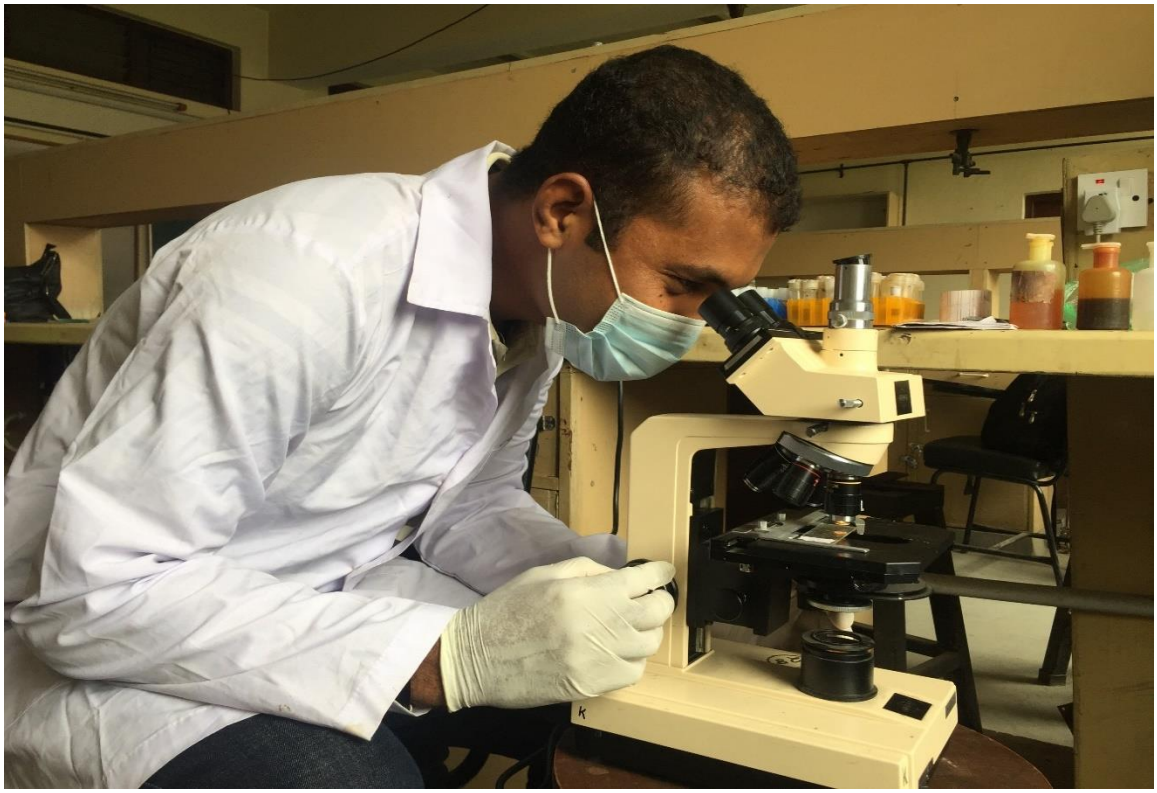

Figure 17: Sample examination

3.3.6.7 Identification of eggs

Eggs of parasites were identified on the basis of shape, size and colour of eggs and with the help of book (Zajac and Conboy, 2012) and other published articles.

3.3.7 Statistical analysis

Data collected from the field was entered into an Excel spredsheet. The statistical package R version 3.3.1 (2016-06-21) was used for analysis by importing the data from Excel. Pearson's Chi-squared test was used to test for significance in parasitic load among the two species of bats.

3.3.8 Ethical, Legal and Social Implications (ELSI)

Recommendation letter from Central Department of Zoology and legal permission from Department of Forestry and its sectors were used to allow research in Ghangre Community Forest and Kalidevi Community Forest of Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

4 RESULTS

4.1 Species composition

A total of 44 bats belonging to two families and two genera, *Megaderma lyra* commonly called greater false vampire bat (N=26) and *Eonycteris speleae* commonly called dawn bat (N=18) from Chamero Cave and Chamero Thok respectively were captured and examined.

Table 1: Prevalence of ectoparasites in bats of Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Host	Total bat captured	Prevalence (%)	Habitat	GPS location
Megaderma lyra	26	100	Rocky cave crevices Chamero Cave	N27^{0}49.112' E84^{0}58.24'
Eonycteris spelaea	18	88.88	Rocky cave crevices Chamero Thok	N 27 ^{o} 49.211' E 84 ^{o} 58.578'

4.2 Ectoparasites of bats

Spinturnix sp.

The specimens were collected from all over the body of *Eonycteris spelaea* and *Megaderma lyra* at Chamero Cave and Chamero Thok, Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Identifying key upto Genus

1. With four pairs of legs (most Acarina).
2. Not as above, body length less than 2 mm (mites, other than ticks and chiggers).
3. With peritreme and lateral stigma dorsal to and at level between coxae Ill and IV (Mesostigmatid mites).
4. Ventral shields reduced.
5. Not particularly tick-like, chelicerae small; tritosternum absent or represented by small basal remnant..... Spinturnicidae.
6. Postanal seta present ; 5 pairs of setae surrounding anterior margin of dorsal shield; a row of setae posterior to stigmata ; tritosternum reduced ; dorsal peritreme long, with anterior end bending ventrad and reaching ventral surface between coxae II & III ..... ***Spinturnix***.

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Spinturnix sp. (10X)

Generic characters

Members of this genus have long, dorsal peritreme, anterior end bending ventrad and reaching ventral surface of body between coxae II & III. Four or more pairs of setae surrounding anterior margin of dorsal shield; few to several pairs of marginal setae posterior to stigmata. Tritosternu small. Sternal plate usually reticulate. Postanal seta present. Legs more or less equal in size, claws of leg I not enlarged, and caruncles of leg I well developed. Striae of body either possess lobes, or have a strongly shingled appearance, or form an interweavng design.

Ancystropus sp.

The specimens were collected from all over the body of *Eonycteris spelaea* at Chamero Thok, Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Identifying key upto Genus

1. With four pairs of legs (most Acarina).
2. Not as above, body length less than 2 mm (mites, other than ticks and chiggers).
3. With peritreme and lateral stigma dorsal to and at level between coxae Ill and IV (Mesostigmatid mites).
4. Ventral shields reduced.
5. Not particularly tick-like, chelicerae small; tritosternum absent or represented by small basal remnant..... Spinturnicidae
6. Postanal seta lacking ; 2-4 pairs of setae surrounding anterior margin of dorsal shield ; a pair of setae below stigmata ; dorsal peritreme short, lying over coxa III, not bending ventrad; tritosternum large.
7. Claws I greatly enlarged ; caruncles I rudimentary; usually 2-3 pairs of setae sur- rounding anterior margin of dorsal shield; distal setae on tarsus I slender.
8. Three pairs of setae surrounding anterior margin of dorsal shield ; epigynial shield located at level of coxa IV; without lateral hooks on tarsus I and coxa I... ***Ancystropus***

Ancystropus sp. (10X)

Generic characters

Leg I, distinct and enlarged, with strong ventral hooks ; claws I large, and car-uncles I are rudimentary. There are 3 pairs of setae surrounding anterior margin of dorsal shield; peritreme short; epigynial shield located at level of coxa IV; and anal shield lacks postanal seta. Legs have short to very short setae; distal setae on tarsus I slender. Trito- sternum characteristic in that anterior corners project toward gnathosoma.

Trichobius sp.

The specimens were collected from all over the body of *Eonycteris spelaea* and *Megaderma lyra* at Chamero Cave and Chamero Thok, Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Identifying key upto family

1. With three pairs of legs (insects, chiggers and larval ticks).
2. Winged.
3. One pair of wings.....,.....Streblidae

Generic characters

Presence of three pairs of legs. One pair of winged. Abdominal ctenidia is absent. Abdominal fusiform and shrunken.

Trichobius sp. (10X)

Ischnopsyllus sp.

The specimens were collected from all over the body of *Eonycteris spelaea* and *Megaderma lyra* at Chamero Cave and Chamero Thok, Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Identifying key upto family

1. With three pairs of legs (insects, chiggers and larval ticks).
2. Not winged.
3. Strongly laterally flattened.
4. Two tooth-like spines on anterior portion of head (genal comb); legs adapted for jumping .....Bat fleas, lschnopsyllidae

Generic characters

Presence of three pairs of legs. Not winged. Body is laterally flattened. Mouth parts constitute of two tooth-like spines on anterior portion of head (genal comb). Legs adapted for jumping. Abdominal ctenidia present and abdomen fusiform in shape.

Ischnopsyllus sp. (10X)

Basilia sp.

The specimens were collected from all over the body of *Eonycteris spelaea* at Chamero Thok, Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Identifying key upto family

1. With three pairs of legs (insects, chiggers and larval ticks).
2. Not winged.
3. Strongly laterally flattened.
4. No such teeth, legs not adapted for jumping ……..Nycteribiidae

Basilia sp. (10X)

Generic characters

Presence of three pairs of legs. Not winged. Body is laterally flattened. Two tooth like spines on anterior portion of head (genal comb) is absent. Legs not adapted for jumping. Abdominal ctenidia present. Abdomen oval. The arrangement and chaetotaxy of the female abdominal sclerotized plates. The only difference, viz., the absence of eyes, is by no means good generic character.

Table 2: Species wise prevalence of ectoparasites of bats of Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Bat Family and species	**Sample size**	**Ectoparasites Family and species**	**Number of bat infested**	**Prevalence (%)**
Megadermatidae	26	Streblidae		
Megaderma lyra		*Trichobius* sp.	26	100
		Ischnopsyllidae		
		Ischnopsyllus sp.	2	7.69
		Spinturnicidae		
		Spinturnix sp.	5	19.23
Pteropodidae	18	Streblidae		
Eonycteris spelaea		*Trichobius* sp.	4	22.22
		Ischnopsyllidae		
		Ischnopsyllus sp.	1	5.55
		Nycteribiidae		
		Basilia sp.	16	88.88
		Spinturnicidae		
		Spinturnix sp.	4	22.22
		Ancystropus sp.	5	27.77

The *Megaderma lyra* was found to harbor a three species of ectoparasites *Trichobius* sp., *Ischnopsyllus* sp. and *Spinturnix* sp. Predominant ectoparasites includes *Trichobius* sp. (bat flies) which were found in all captured bats while *Ischnopsyllus* sp. (bat fleas) and *Spinturnix* sp. (mites) were found in two and five bats respectively (Table: 2).

The *Eonycteris speleae* was found to harbor five species of ectoparasites *Trichobius* sp., *Ischnopsyllus* sp., *Spinturnix* sp., *Basilia* sp. *and Ancystropus* sp. Predominant ectoparasites includes *Basilia* sp. (bat louse) which were found in sixteen captured bats while *Ischnopsyllus* sp., *Spinturnix* sp., *Trichobius* sp. *and Ancystropus* sp. were found one, four, four and five respectively (Table 2).

Table 3: Association of parasitic infestation of bats of Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Ectoparasites	*Megaderma lyra*(26)	*Eonycteris speleae*(18)	χ^2	P-value
***Trichobius* sp.**	26	4	26.182	3.106e-07
***Ischnopsyllus* sp.**	2	1	4.4029e-31	1
***Basilia* sp.**	0	16	32.578	1.145e-08
***Spinturnix* sp.**	5	4	3.9036e-31	1
***Ancystropus* sp.**	0	5	5.6237	0.01772

Association of ectoparasite infestation in two bat species were statistically analyzed. Both species of bats were found almost equally infested with *Ischnopsyllus* sp. *Spinturnix* sp. of flea and mites respectively with insignificant distribution. Regarding *Trichobius* sp., *Megaderma lyra* of Chamero Cave were significantly higher infestation compared *Eonycteris speleae* of Chamero Thok. But with respect to *Basilia* sp. and *Ancystropus* sp. which were found only in *Eonycteris speleae* (Table 3).

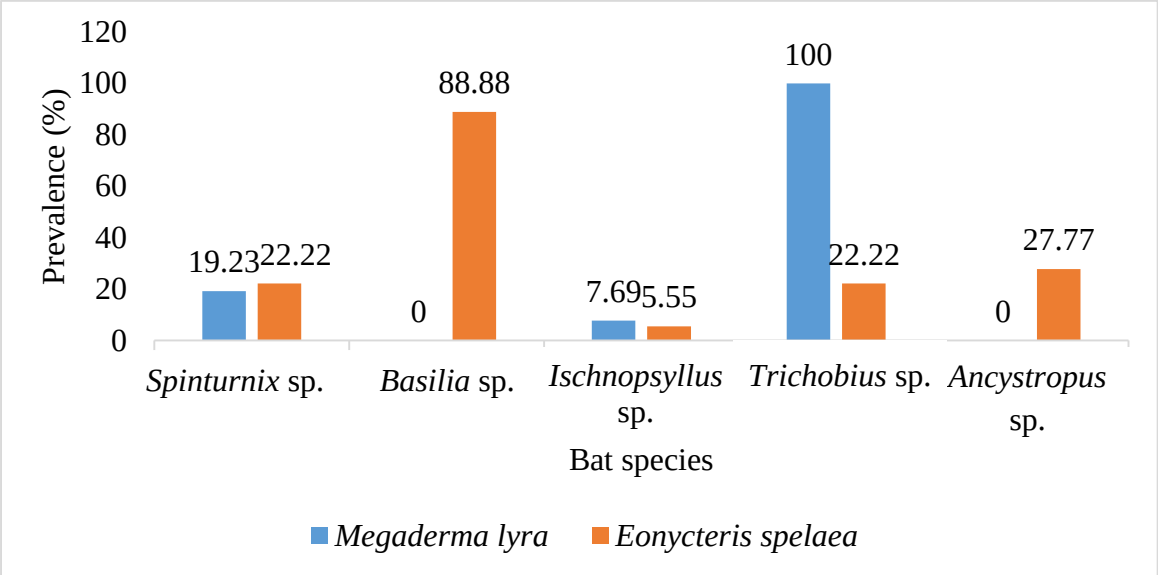

Figure 18: Ectoparasitic infestation of bat species in Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Prevalence wise comparison of ectoparasitic infestation showed that *Basilia* sp. was found highly infested in *Eonycteris speleae* of Chamero Thok. Similarly, *Trichobius* sp. was found highly infested in *Megaderma lyra* of Chamero Cave (Figure 18).

4.3 Endoparasites of bats

Bat species of both study area were found to be infected with high prevalence of endoparasites. Prevalence of endoparasites in *Eonycteris speleae* (29.54%) was found slightly higher than in *Megaderama lyra* (25%) (Table 4).

Table 4: Prevalence of endoparasites in bats of Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Host	Total fecal samples	Prevalence (%)	Habitat	GPS location
Megaderma lyra	16	25	Rocky cave crevices Chamero Cave	N27^{0}49.112' E084^{0}58.24'
Eonycteris spelaea	44	29.54	Rocky cave crevices Chamero Thok	N 27 ^{o} 49.211' E 84 ^{o} 58.578'

Table 5: Species wise prevalence of endoparasites in bats of Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Bat Family and species	Sample size	Endoparasites	Number of infected bats	Prevalence (%)
Megadermatidae ***Megaderma lyra***	16	*Hymenolepis* sp. Ascarid Hookworm	1 1 2	6.25 6.25 12.5
Pteropodidae ***Eonycteris spelaea***	44	*Hymenolepis* sp. Ascarid Hookworm Strongylid	3 6 3 1	6.82 13.64 6.82 2.27

The *Megaderma lyra* was found to be infected with three species of endoparasites *Hymenolepis* sp., ascarid and hookwoom. Among the 16 feacal samples, hookworm was found high prevalence (12.5%) than *Hymenolepis* sp. and ascarid (6.25%) (Table 5).

The *Eonycteris speleae* was found to be infected with four species of endoparasites *Hymenolepis* sp., ascarid, hookworm and strongylid. Among the 44 feacal samples, ascarid was found high prevalence (13.64%) and strongylid was found low prevalence (2.27%) among four endoparasites (Table 5).

Table 6: Association of parasitic infection in bats of Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Endoparasites	*Megaderma lyra*(16)	*Eonycteris speleae*(44)	χ^2	P-value
***Hymenolepis* sp.**	1	3	3.1022e-31	1
Ascarid	1	6	0.11119	0.7388
Hookworm	2	3	0.030992	0.8603
Strongylid	0	1	6.8853e-32	1

Eonycteris speleae was found to be infected with higher prevalence of all four species of endoparasites compared to *Megaderma lyra. Hymenolepis* sp., ascarid and hookworm were found in both bats species with statistically insignificant distribution. While strongylid was found only in *Eonycteris speleae* (Table 6).

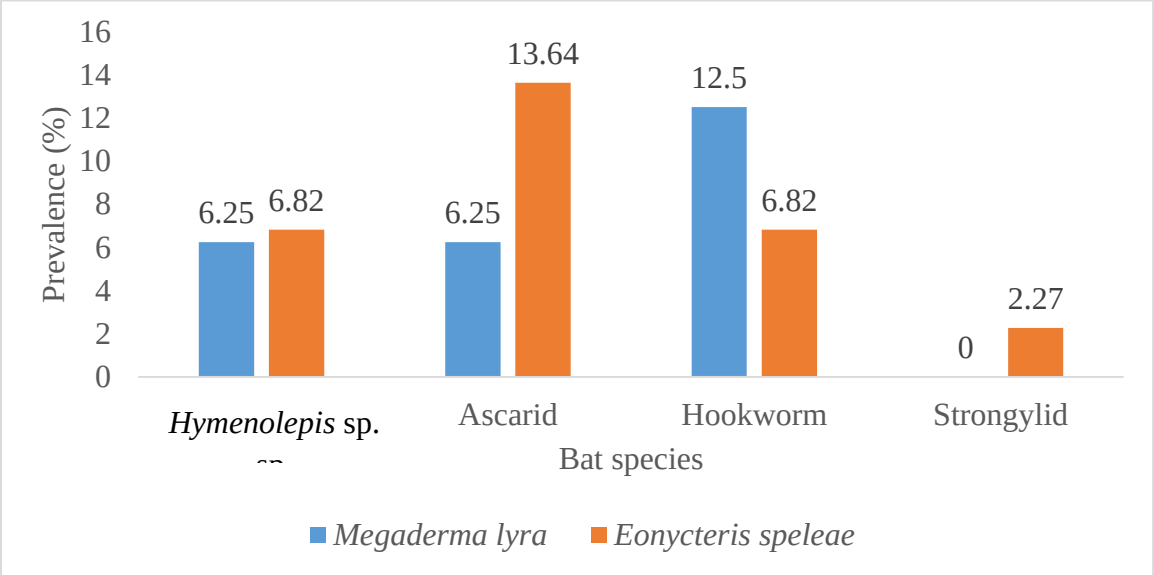

Figure 19: Endoparasitic infection of bat species in Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Prevalence wise comparison of endoparasitic infection showed that ascarid was found slightly higher in *Eonycteris speleae*, while hookworm was found slightly higher in *Megaderma lyra,* likewise *Hymenolepis* sp. was found almost same prevalence in both bat species *Megaderma lyra* and *Eonycteris speleae* and strongylid was absent in *Megaderma lyra* (Figure 19).

Table 7: Endoparasitic intensity of bats of Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Host	Endoparasites	Prevalence	Intensity		
			Low	Mild	High
Megaderma lya	*Hymenolepis* sp.	6.25%	1	-	-
	Ascarid	6.25%	1	-	-
	Hookworm	12.5%	1	1	-
Eonycteris speleae	*Hymenolepis* sp.	6.82%	2	1	-
	Ascarid	13.64%	5	1	-
	Hookworm	6.82%	1	2	-
	Strongylid	2.27%	1	-	-

Among 16 feacal samples of *Megaderma lyra,* four samples were found positive. Three samples each having *Hymenolepis* sp., ascarid and hookworm were low intensity while one sample with hookworm was found infected with mild intensity.

Among 44 feacal samples of *Eonycteris speleae,* thirteen samples were positive. Two samples having *Hymenolepis* sp., five samples having ascarid and one sample having each hookworm and strongylid were found low intensity. While two samples having hookworm and one sample having each of *Hymenolepis* sp. and ascarid were found mild intensity.

Notes:

Low intensity <2 eggs

Mild intensity 2- 4 eggs

High intensity >4 eggs

5 DISCUSSION

Taxonomic studies of bat ectoparasites were carried out on the basis of morphological characteristics. Till date bats have been reported to be infested with bat flies, bat louse, bat fleas, mites and ticks. Among them the available literature showed that bat flies and mites were most common ectoparasites of bats. According to available literature, the common ectoparasites of bats were *Trichobius costalimai, T. angulatus, T. adamsi, T. sphaeronotus, T. costalimai, T. tiptoni, T. johnsonae, Strebla curvata, S. hertigi, Trichobioides perspicillatus, Raymondia lobulata* (Streblidea), *Basilia lindolphoi, B. mimoni, B.antrozoi, B.corynorbini, B. frocipata, B.* new sp., *Nycteridopsyllia ancyluris, N. dictena, N. eusarca, N. longiceps, N. pentactena, Rhinolophopsylla unipectinata* (Nycteribiidae), *Ischnopsyllus elongatus, I. hexactenus, I. intermedius, I. mysticus, I. obscurus, I. octactenus, I. simples, I. variabilis, Myodopsylla collinsi* (Ischnopsyllidae), *Spinturnix carlashoffmanni, S. americanus, Ancystropus* sp. (Spiturnicidae) (Lukoschus *et al.* 1973; Fritz 1983; Autino *et al.* 2011; Nartey 2015). In present study, *Trichobius* sp. (Streblidae), *Ischnopsyllus* sp. (Ischnopsyllidae) and *Spinturnix* sp. (Spinturnicidae) were found in *Megaderma lyra* of Chamero Cave, Kalleri, Galchhi Rural Municipality, Dhading, Nepal and *Trichobius* sp. (Streblidae), *Ischnopsyllus* sp. (Ischnopsyllidae), *Spinturnix* sp. and *Ancystropus* sp. (Spinturnicidae) and *Basilia* sp. (Nycteribiidae) were found in *Eonycteris speleae* of Chamero Thok, Kalleri, Galchhi Rural Municipality, Dhading, Nepal.

Bat flies (*Trichobius sp.*) are the most common ectoparasites of *Megaderma lyra* of Chamero Gupha, Kalleri, Galchhi Rural Municipality, Dhading followed by mites and bat louse (*Basilia sp.*) are most common ectoparasites of Chamero Thok, Kalleri, Galchhi Rural Municipality, Dhading followed by two species of mites (*Ancystropus sp.* and *Spinturnix sp.*) and bat flies (*Trichobius sp.*). This data corroborate with the study of Autino *et al.* (2011) who reported that mites and bat flies were common ectoparasites on bats, where bat fleas are rarely observed on bats. Many studies also showed that bat flies and mites were the most abundant ectoparasites of bats. Moras, *et al.* (2011) found 14 bat fly species and 9 mite species from bats in southern Minas Genais, Brazil. Almeida *et al.* (2011) reported 10 bat fly species and 11 mite species from bats in southeastern Brasil. Ferenc and Myslajek (2003) discovered 8 species of mites (including one with two subspecies) in Polland. Wood (2012) reported four species of flies and eight species of mites in *Miniopterus natalensis* in South Africa. More than 36500 bat flies of 116 species were captured from more than 25000 bats representing 130 species in Venezuela (Dick and Patterson 2008). Obame-Nkoghe *et al. (*2016) reported 439 bats infested by batflies in Gabon, out of 1,154 bats. Maa (1971) recorded 16 streblid species of the megachiroptera. Hofstede *et al.* (2004) collected 773 ectoparasitic bat flies on 455 bats from the Lamanai area of Belize. Kristofik and Danko (2012) found 14 species of the family Spinturnicidae with two subspecies belonging to three genera: *Paraperiglischrus, Eyndhovenia* and *Spinturnix.* Wilson and Wodzicki (1977) reported the mite *Meristaspis calcarata* from *Pteropus t. tonganus* Quay and Gaimard in the Pacific region. The sixteen species of streblid bat flies were collected from 10 bat species of the family Phyllostomidae where thirteen of these streblid species were recorded for the first time in Pernambucu, Brazil (Soares *et al.*, 2008). Krichbaum *et al.*

(2009) found ectoparasites as bat flies of a previously undescribed species of the *Nycterophilia* genus from all three species of bats *Mormoops blainvillei, Pteronotus quadridens* and *Monophyllus redmani* from Puerto Rico. Fritz (1983) found female bats were significantly higher numbers of bats flies in Hawaii, USA and *Trichobius joblingi, Speiseria ambigua* and *Strebla guajiro* were found on *Carollia.* Graciolli and Azevedo (2011) found ectoparasites of bats, with a description of a new species of Synthesiostrebla Townsend (Diptera, Streblidae) from Brazil.

Similarly, Delfinado and Baker (1963) reported four genera in Spinturnicidae from bats collected in the Philippines. A total of 1,259 specimens from six species in the Streblidae family were collected from 332 bats in caves within the Cafuringa Environmental Protection Area in the Federal District of Brazil (Souza and Antonini, 2011). Dick and Gettinger (2005) collected 2,467 bat flies representing 11 genera and 31 nominal species of Streblidae, of which 6 genera and 24 species were new records for Paraguay. Foster and Mertins (1996) reported eight species of mites with *Chiroptonyssus robustipes* being most prevalent (100%) and the mites *Ewingana longa, Dentocarpus macrotrichus* and *Notoedres (Bakeracarus) sp.* were reported for the first time infesting bats from Florida. Guzman-Cornejo *et al.* (2003) reported 12 mite from the states of Puebla, Zacatecas and Durango. Alvarez *et al.* (2016) reported five species of Streblidae from 11 bat species in Naujan Lake National Park, Mindoro Oriental Proviece, Philippines. Uchikawa and Kobayashi (1978) found 12 species of fur-mites belonging to the family Myobiidae and 16 larvae of Argasidae in Thailand. Ferenc and Myslajek (2003) discovered 8 species of mites (including one with two subspecies) in Polland. Jaunbauere *et al.* (2008) found *Spinturnix vespertilionis* and *Nycteribia pedicularia* on sixteen species of bats in Latvia. Lukoschus *et al.* (1973) found one new species (*Psorergatoides glossophagae)* of mite in Surinam bats in Venezuela. Lukoschus *et al.* (1973) found one new species (*Psorergatoides glossophagae)* of mite in Surinam bats in New Guinea. Similarly, (Nyteribosca). Nartey (2015) reported four species of bat flies and eithteen species of mites and the most abundant ectoparasite group found on bats were mites; where the species most frequently encountered were *Spinturnix Americana, Carpoglyphus sp* and *Ancystropus zeleborii* in Ghana. Yunker (1958) examined for parasitic mites in eastern United States and found Spinturnicidae, Dermanyssidae and Trombiculidae. Silva and Graciolli (2013) found nine genera and 11 species of bats were parasitized with *Periglischrus torrealbai, Periglischrus tonatii* and *Periglischrus iheringi* from Pantanal, Brasil. Gordon and Ross (2010) surveyed 20000 bats of various species for the parasites in Arizona and found Streblidae as major parasites. These data confirm that bat flies and mites are the primary ectoparasites of several bat species.

Rupp *et al.* (2004) reported 33 species of eight parasitic families of fleas (Ischnopsyllidae), batflies (Nycteribiidae), bugs (Cimicidae), mites (Spinturnicidae, Macronyssidae, Trombiculidae, Sarcoptidae) and ticks (Argasidae, Ixodidae) in Bavaria, Germany. Rupp *et al.* (2004) reported 33 species of eight parasitic families of fleas (Ischnopsyllidae), batflies (Nycteribiidae), bugs (Cimicidae), mites (Spinturnicidae, Macronyssidae, Trombiculidae, Sarcoptidae) and ticks (Argasidae, Ixodidae) in Bavaria, Germany. Orlova (2011) reported twenty species belonging to 8 families of insects and mites from six bat species in northern Eurasia. Valdez *et al.* (2009) recorded 10 families and 13 genera of

insect or acari as the major species mites *Macronyssus crosbyi, Alabidocarpus calcaratus, Acanthophthirius lucifugus* and *Alabidocarpus nr. Eptesicus* in 634 fresh hosts and 1524 museum specimens of bats from Mexico and southern Colorado. Alvarez *et al.* (2016) reported seven species of Nycteribiidae and one species of Ischnopsyllidae (Siphonaptera) from 11 bat species in Naujan Lake National Park, Mindoro Oriental Proviece, Philippines. Two hundred twenty five ectoparasites representing 36 species and seven families were collected from 140 bats captured in mount makiling, Laguna, Philippiness (Alvarez, 2013). Dahal and Thapa (2010) had reported each specimen of Cimicidae and Ischnopsyllidae, two specimens of Spinturnicidae and fifteen specimens each of Nycteribiidae and Streblidae ectoparasites associated with bats from Kathmandu Valley. In present study, the *Megaderma lyra* was found to harbor a three species of ectoparasites *Trichobius* sp., *Ishnopsyllus* sp. and *Spinturnix* sp. Predominant ectoparasites includes *Trichobius* sp. (bat flies) which were found in all captured bats while *Ishnopsyllus* sp. (bat fleas) and *Spinturnix* sp. (mites) were found in two and five bats respectively and The *Eonycteris speleae* was found to harbor large number of ectoparasites *Trichobius* sp., *Ishnopsyllus* sp., *Spinturnix* sp., *Basilia* sp. *and Ancystropus* sp. Predominant ectoparasites includes *Basilia* sp. (bat louse) which were found in sixteen captured bats while *Ishnopsyllus* sp., *Spinturnix* sp., *Trichobius* sp. *and Ancystropus* sp. were found one, four, four and five respectively.

Aroon *et al.* (2015) found 31.82% of bats were infested with ectoparasites belonging to 4 families, 7 genera and 7 species in northeastern Thailand. Similarly, Dick *et al.* (2003) found 13.2% ectoparasitic arthropods of four species of the host individuals at Pennsylvania's Canoe Creek State Park. Of 181 bats belonging to 16 species, 34.1% were infested by streblid flies and 33.5% by spinturnicid mites and 8.3% by macronyssid mites in southeastern Brazil. (Almeida *et al.,* 2011). In this present study, *Megaderma lyra* was found 100% prevalence with 3 families, 3 genera and 3 species of ectoparasites while *Eonycteris speleae* was found 88.88% prevalence with 4 families, 5 genera and 5 species of ectoparasites. By comparing with Aroon *et al.* (2015), Dick *et al.* (2003) and Almeida *et al.* (2011), present study shows high infestation of ectoparasites in bats.

Generally bats can be affected with both ecto and endoparasites. According to available literature the major endoparasites reported in bats are *Eimeria antrozon, Eimeria hessei, Biacantha normaliae Nycteria* spp., Allossogonoporus *sp., Acanthatrium eptesici, A. microcanthum, A. oligacanthum, A. pipistrelli,Paralecithodendrium transversum, Ochoterenatrema diminutum, Anenterotrema eduardocaballeroi, Anenterotrema liliputianum, Ochoterenatrema caballeroi, Tricholeiperia sp., Parahistrostrongylus octacanthus, Litomosoides guiterasi, Litomosoides brasiliensis, Capillariinase* sp., *Hymenolepididae* sp., hookworm, *Litomosa chiropterorum, Molinostronglylus orhatus, Hasstilesia tricolor, Plagiochis* sp., *Mesotretes peregrinus, Lecithodendrium, Pyenoporus heteroporus, Prosthodendrium parvouterus, Prosthodendrium corberensis,* (Trematode), *Vampirolepis elongates, Hymenolepis grisea, Hymenolepidid* (Cestodes), *Cheiropteronema globocephala, Capillaria* sp., *Strongylacantha glycirrhiza, Litomosa attavianii, Seuratum cancellatum, Ascarididae* sp., *Physaloptera brevivaginata* (Nematodes) (Gay *et al.,* 2014; De Albuquerque *et al.,* 2016; Mcallister *et al.,* 2007; Heddergott and Steinbach 2015; Lotz and Font 1983).

The overall prevalence of endoparasites of *Megaderma lyra* (25%) and *Eonycteris speleae* (29.54%) were slightly higher as compared to the prevalence rates of 22% and 20.89% obtained by the previous studies (Gay *et al.*, 2014 and De Albuquerque *et al.*, 2016) respectively. But it was lowered than 60%, 88.2%, 76% and 43.5% obtained by Pistole 1988, Horvat *et al.*, 2015, Lord *et al.*, 2012 and Saoud and Ramadan 1976 respectively.

Wood (2012) reported 11 helminth (one cestode, seven nematode and three trematode) species in *Miniopterus natalensis* in South Africa. Similarly, Hilton and Best (2000) found bats were infected with 37.3% helminthes, 4.3% cestodes, 14.5% nematodes and 23.8% trematodes and 4.6% were parasite by two types of helminths and one had all three types of helminthes in Alabama. Mcallister *et al.* (2004) found twelve lecithodendrid trematodes, *Prosthodendrium transversum* 20% and seven ascarid nematodes, *Seuratum cancellatum* 20% were found in small intestine of *Myotis septentrionalis* and ova of nematode *Capillaria palmate* 22% in the feces of *Eptesicus fuscus* from Pipistrelle Mine, Polk County, Arkansas. Williams (1957) reported total of twelve species and one subspecies of helminths, including two new species representing one cestode (*Hymenolepis* roudabushi), nine trematodes (*Prosthodendrium transversum, P. ascidia navicula, Acanthatrium microcanthum, A. eptesici, A. lunatum sp., A. obovatum. sp., Acanthatrium* sp., *Limatulum gastroides* and *Plagiorchis micracanthos*) and three species of nematoda (*Physocephalus sexalatu, Ascarops strongylina* and *Capillaria sp.*) from bats in United States. In present study, The *Megaderma lyra* was found to be infected with three species of endoparasites *Hymenolepis* sp., ascarid and hookwoom. Hookworm was found high prevalence (12.5%) than *Hymenolepis* sp. and ascarid (6.25%). Similarly, The *Eonycteris speleae* was found to be infected with four species of endoparasites *Hymenolepis* sp., ascarid, hookworm and strongylid. Ascarid was found high prevalence (13.64%) and strongylid was found low prevalence (2.27%) among four endoparasites.

Lotz and Font (1983) found fifteen species of Lecithodendriidae from *Eptesicus fuscus* in Wisconsin and Minnesota: *Acanthatrium eptesici, A. microcanthum, A. oligacanthum, A. pipistrelli, Allassogonoporus marginalis, Glyptoporus noctophilus, Ochoterenatrema breckenridgei, O. diminutum, O. travassosi, Paralecithodendrium chilostomum, P. macnabi, P. naviculum, P. nabiculum, P. nokomis, P. swansoni* and *P. transversum.* Similarly, Krichbaum *et al.* (2009) found cestode *Vampirolepis christensoni* and nematode *Capillaria pusilla* from Puerto Rico. Esteban *et al.* (2001) found five species of trematodes, *Plagiorchis* (*Plagiouchis*) sp., *Lecithodendrium* (*Lecithodendrium*) *linstowi, Prosthodendrium* (*Prosthodendrium*) sp., *Pycnoporus heteroporus* and *Parabascus semisquasus* and species of cestode, *Hymenolepis pipistrelli* in bats from Spain. . Mcallister (2008) found two plagorchiid trematodes in small intestine of *Myotis ciliolabrum* in south Dakota. Both trematodes were identified as *Plagiorchis microcanthos* (Macy, 1931). Mcallister *et al.* (2007) found the bats were infected with lecithodendrid trematodes, *Acanthatrium alicatai* and nematodes (*Seuratum cancellatum)* and acoccidian (*Eimeria antrozon)* in Southwestern Texas. While in present study, *Megaderma lyra* was found to be infected with three species of endoparasites *Hymenolepis* sp., ascarid and hookwoom and *Eonycteris speleae* was found to be infected with four species of endoparasites *Hymenolepis* sp., ascarid, hookworm and stronglylid.

Foster and Mertins (1996) found eleven species of helminthes parasites which included 7

trematodes, 3 nematodes and 1 cestode, all of which were new records for bats from Florida where *Vampirolepis decipiens* and *Ochoterenatrema breckenridgei* was first record for *Tadarida brasiliensis* in the United States and the nematodes *Molinostrongylus delicatus* was the most prevalent helminth collected (64%). Similarly, Guzman-Cornejo *et al.* (2003) examined ninety-eight Mexican free-tailed bats, *Tadarida brasiliensis mexicana* and found 5 helminth taxa (3 digenean, 1 cestode and 1 nematode) from the states of Puebla, Zacatecas and Durango. Matskasi (1973) examined 278 bats specimens representing 36 species collected in India, 22 trematode species were identified and three new species (*Neoheterophyes topali, Prosthodendrium robustum* and *Parabascus ghati*) were described. Sawada (1970) examined three species of common Japanese cave-bats, *Rinolophus ferrumequinum, R. cornutus* and *Miniopterus schreibersi* in Okayama Prefecture for the presence of cestodes and found eleven of these twenty-four bats harbored five species of hymenolepid cestodes, of which were new ones and the cestodes were identified as *Insectivorolepis okamotoi* sp., *Vampirolepis isensis, Rodentolepis mocrotesticulatus* sp. and *Insectivorolepis niimiensis* sp. In present study, two bats were infected with hookworm and one each bat were infected with *Hymenolepis* sp. and ascarid in case of *Megaderma lyra.* While six bats were infected with ascarid, one bat was infected with strongylid and three bats were infected with *Hymenolepis* sp. and hookworm in case of *Eonycteris speleae.*

6 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The findings of the study indicated that bats indeed harbor a wide array of ectoparasites (bat flies, mites, bat fleas and bat louse) and endoparasites. Two species of mites and one species of each bat flies, bat fleas and bat louse were collected from the 44 bats captured. Bat flies were the most abundant ectoparasite of *Megaderma lyra* while bat louse was the predominant ectoparasite of *Eonycteris speleae.*

Ectoparasites were collected from two study sites. In Chamero Cave, the *Megaderma lyra* had three species of ectoparasites and In Chamero Thok, the *Eonycteris speleae* had five species of ectoparasites. Both bats were found almost equally infested with *Ischnopsyllus* sp. *Spinturnix* sp. of flea and mites respectively with insignificant distribution. Regarding *Trichobius* sp., *Megaderma lyra* of Chamero Cave were significantly higher infestation compared *Eonycteris speleae.* But with respect to *Basilia* sp. and *Ancystropus* sp. which were found only in *Eonycteris speleae.* Prevalence wise comparison of ectoparasitic infestation showed that *Basilia* sp. was found highly infested in *Eonycteris speleae* of Chamero Thok. Similarly, *Trichobius* sp. was found highly infested in *Megaderma lyra* of Chamero Cave.

Bat species of both study area were found to be infected with high prevalence of endoparasites. Prevalence of endoparasites in *Eonycteris speleae* (29.54%) was found slightly higher than in *Megaderama lyra* (25%). The *Megaderma lyra* was found to be infected with three species of endoparasites *Hymenolepis* sp., ascarid and hookwoom. Hookworm was found high prevalence (12.5%) than *Hymenolepis* sp. and ascarid (6.25%). While the *Eonycteris speleae* was found to be infected with four species of endoparasites *Hymenolepis* sp., ascarid, hookworm and strongylid. Ascarid was found high prevalence (13.64%) and strongylid was found low prevalence (2.27%) among four endoparasites.

Eonycteris speleae was found to be infected with higher prevalence of all four species of endoparasites compared to *Megaderma lyra. Hymenolepis* sp., ascarid and hookworm were found in both bats species with statistically insignificant distribution. While strongylid was found only in *Eonycteris speleae.* Prevalence wise comparison of endoparasitic infection showed that ascarid was found slightly higher in *Eonycteris speleae,* while hookworm was found slightly higher in *Megaderma lyra,* likewise *Hymenolepis* sp. was found almost same prevalence in both bat species *Megaderma lyra* and *Eonycteris speleae* and strongylid was absent in *Megaderma lyra.*

In summary, bats were noted to harbor parasites (bat flies, mites, bat louse, bat fleas and helminths) of noticeable public health importance.

6.2 Recommendations

- ❖ The present study has attempted upto genera, the further study is required for taxonomic details upto species level.
- ❖ The study of endoparasites is first attempt in Nepal. The detailed study is required for further information.
- ❖ Further studies are required to shed light on the importance of these parasitic infestaions to public health and biodiversity conservation efforts.
- ❖ Additional investigations are required to determine the vectoral role of bat ectoparasites in disease transmission to humans and animals which have zoonotic importance.

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