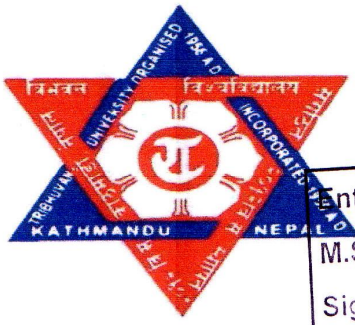
GASTROINTESTINAL PARASITES OF KADAKNATH CHICKEN (*Gallus gallus domesticus* Linnaeus, 1758) AND CROSS BREED KADAKNATH IN SUDDODHAN RURAL MUNICIPALITY, RUPANDEHI, NEPAL



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T.U. Redg. No: 5-2-448-52-2011

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Batch: 2073/74

A thesis submitted

In partial fulfillment of the requirement for the award of the degree of Master of Science in Zoology with special paper Parasitology

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

September, 2019

DECLARATION

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

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RECOMMENDATION

This is to recommend that the thesis entitled **"GASTROINTESTINAL PARASITES OF KADAKNATH CHICKEN (*Gallus gallus domesticus* Linnaeus, 1758) AND CROSS BREED KADAKNATH IN SUDDODHAN RURAL MUNICIPALITY, RUPANDEHI, NEPAL"** has been carried out by Mrs. Ganga Khadka for the partial fulfillment of Master's degree of Science in Zoology with special paper in Parasitology. This is her 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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LETTER OF APPROVAL

On the recommendation of supervisor Prof. Dr. Mahendra Maharjan, this thesis submitted by Mrs. Ganga Khadka entitled **"GASTROINTESTINAL PARASITES OF KADAKNATH CHICKEN (*Gallus gallus domesticus* Linnaeus, 1758) AND CROSS BREED KADAKNATH IN SUDDODHAN RURAL MUNICIPALITY, RUPANDEHI, NEPAL"** is approved for the examination and submitted to Tribhuvan University in Partial fulfillment of the requirement for Master's Degree of Science in Zoology with special paper in Parasitology.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Mrs Ganga Khadka entitled **"GASTROINTESTINAL PARASITES OF KADAKNATH CHICKEN (*Gallus gallus domesticus* Linnaeus, 1758) AND CROSS BREED KADAKNATH IN SUDDODHAN RURAL MUNICIPALITY, RUPANDEHI, NEPAL"** has been accepted as a partial fulfillment for the requirement of Master's Degree of Science in Zoology with special paper in Parasitology.

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ACKNOWLEDGEMENTS

First and foremost, I am greatly thankful to my supervisor Professor Dr. Mahendra Maharjan of Central Department of Zoology, Kirtipur, Kathmandu for his considerable guidance, appropriate supervision, constant encouragement and valuable suggestions during research work.

I am deeply indebted to Prof. Dr. Tej Bahadur Thapa, Head of the Central Department of Zoology for his valuable suggestion and provision of required departmental facilities to complete my study work.

I express my deepest appreciation to Mr. Mahendra Gautam , proprietor of Gautam Poultry Farm, who gave me chance to complete my dissertation work in his farm. I also thank to the staff of farm who helped me during the sample collection.

I am extremely thankful and grateful to my husband Mr. Laxman Aryal and entire family for constant and priceless advice, encouragement, financial and moral support on everything throughout my work. I am also thankful to all my friends, teachers and staff of Central Department of Zoology for their valuable help during my work.

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LIST OF ABBREVIATIONS

CDZ	Central Department of Zoology
FAO	Food and Agriculture Organization
GI	Gastro-intestinal
i.e.	That is
mm	Milimeter
OECD	Organization for Economic Discussion and Development
P value	Probability value
rpm	Rotation per minute

ABSTRACT

Kadaknath is a native bird of Madhya Pradesh and recently introduced in poultry farming in Belawa, Rupandehi, Nepal whereas cross breed were later introduced in the farm and these are the cross of pure breed Kadaknath and local chicken(New Hampshire breed). A study was conducted to determine the prevalence of gastrointestinal parasites of Kadaknath and cross breed Kadaknath in Suddodhan rural municipality, Rupandehi, Nepal. A total of 150 fecal samples were collected from the farm comprising of Kadaknath (100) and cross breed (50) and examined using concentration methods and 54% of Kadaknath and 44% of cross breed were infected. Three species of nematodes and two species of *Eimeria* from both pure breed and cross breed and only one species of cestode from Kadaknath were recorded. In case of Kadaknath, highest prevalence rate was found infected with *Ascaridia* sp. (35%) followed by *Heterakis* sp. (12%) and *Eimeria tenella* (12%), *Eimeria maxima* (7%), *Raillietina* sp. (4%) and *Trichostrongylus* sp. (2%). *Eimeria tenella* and *Ascaridia s*p. showed the heavy mode of infection in Kadaknath. Whereas in cross breed, highest prevalence rate was found *Ascaridia* sp. (24%) followed by *Heterakis* sp. (20%) and other parasites. Only *Eimeria maxima* (4%) showed heavy mode of infection. Overall comparison between parasites of both the breeds showed higher prevalence in Kadaknath compared to cross breed. Only *Trichostrongylus* sp. nematode parasite was found significantly high infection in cross breed compared to Kadaknath ($\chi 2$ value=4.79, p=0.033) revealing that cross breed are more susceptible to *Trichostrongylus* sp. compared to pure breed. Whereas single and triple mode of infection were found to be statistically significant between these breeds as in pure breed, infection of *Ascaridia* sp. was significantly high ($\chi 2$=10.20, p=0.001). Although both group of chicken are susceptible for various parasitic infection, to prevent the chances of parasitic transfer between them, the flock should be farmed completely separate and both breeds should be treated with antihelminthic and antiprotozoal drugs on a regular basis.

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1. INTRODUCTION

1.1 Background

Poultry farming is often called as allied agriculture industry. Poultry are a domesticated species of birds reared for production of meat, eggs and other allied products like feathers for fancy purpose and excreta as farm manure. Even though term poultry is mostly used for chicken, it also includes other avian species like turkey, duck, guinea fowl and geese. Chicken is also used extensively as a delicious food. The egg is also rich in vitamins like A, D, B_1, B_2 and pantothenic acid (Naber, 1979). According to the report by the Organization for Economic Co-operation and Development (OECD, 2014) and the Food and Agriculture Organization of the United Nation (FAO, 2014) indigenous chicken meat production was 95.8 million and broiler meat production was 108.7 million tones. Indigenous chicken meat production in Nepal was 40.8 thousand tones in 2012. According to FAO, it was seen that in 2014 there was increase in poultry meat production by 1.7 percent and was expected that outcome will expand by more than two percent as it might approach to 98 million tones in 2015 (GPT, 2014). In current intensive poultry production systems, chickens are exposed to a variety of potentially pathogenic organisms like bacteria, virus and parasites.

Kadaknath breed, also known as Kalamashi in Hindi, is known for its black-colored meat. Kadaknath is found in vast areas of Western Madhya Pradesh mainly the Jhabua and Dhar Districts and adjoining areas of Gujarat and Rajasthan (Thakur *et al*.2006). Kadaknath is only Black Meat Chicken (B.M.C.), though the color of the meat is black and unattractive in appearance but it has a delicious flavor (Panda & Mahapatra, 1989). The black color of the flesh is due to the deposition of melanin pigment in the connective tissue and in dermis (Rao & Thomas, 1984).The meat and eggs are considered to be the rich sources of protein and iron (Mohan *et al.* 2008). The Kadaknath birds are resistance to diseases as well as they are also resistant to extreme climatic conditions like summer heat and cold winter stress and can survive in poor sanitation, management (Thakur *et al*.2006)

1.2 Major poultry diseases

Poultry disease are diseases that afflict poultry. The major poultry disease can be broadly categorized as viral disease, bacterial disease and parasitic disease (Sayyed *et al*.2000). Some of the viral diseases in poultry are Avian influenza, Fowl pox, Newcastle disease etc and among them avian influenza and Newcastle disease mostly infect chicken. Avian

influenza also called flu, bird flu, is caused by a virus called H5N1 virus which is released in secretions from the nostrils and in the manure of infected birds and can be spread by the improper disposal of dead birds and manure and by contaminated shoes, clothing and other equipements (Dennis, 2000). Likewise, Newcastle disease is also caused by a virus called paramyxovirus which affects all birds of all ages and can be transmitted through the air within short distances which is characterized by a sudden onset of symptoms which includes hoarse chirps (in chicks), watery discharge nostrils, paralysis, trembling and twisting of the neck (Brown *et al.*1999). Bacterial disease that affect poultry are fowl cholera, infectious coryza etc. Fowl cholera is caused by infection with *Pasteurella multocida* which is mostly found in oral cavity and infectious coryza is an upper respiratory disease of chickens caused by infection with *Haemophilus paragallinarum* (HPG) which is characterized by swollen intraorbital sinuses, nasal discharge and depression which cause significant reduction in the rate of egg production (Glisson, 1998).

1.3 Parasitic disease in poultry

Protozoan parasites of species *Eimeria* is responsible for coccidiosis in poultry. Coccidiosis is one of the most important diseases of chicken in Nepal. On the basis of the organs it affects, the disease is classified as intestinal coccidiosis affecting the small intestine and caecal coccidiosis affecting the large intestine (caeca). At least nine species of *Eimeria* are known to occur in poultry (Jordan and Pattison, 1996). Coccidiosis is characterized by dysentery, enteritis, emaciation, drooping wings, poor growth and low production (Shirzad *et al.*2011). It is considered to be a disease of poor management. The economic importance of the disease is due to its high rate of morbidity and mortality in young birds, reduced feed conversion efficiency and egg production in sub-clinical cases (Rehman *et al.*2010; Awais *et al.*2012). Helminthosis is considered one of the most common diseases that affects free range backyard chickens (Soulsby,1982; Permin *et al.* 1997). Helminthes parasites commonly found in chicken includes Nematodes (roundworms), Cestodes (tapeworms) and Trematodes (flukes). The Nematodes are the most important group of helminthes that affect the chickens both in terms of number and extent of damage caused to the gastro-intestinal tract of the chickens which are *Ascaridia, Heterakis, Capillaria* (Tesfaheywet *et al.*2012). *Ascarid* infections commonly occur in non-caged chickens (Permin & Hansen, 1998). Adults of both *Ascaridia* sp. *a*nd *Heterakis* sp. reside in the small intestine or the caeca respectively. Infection of *Ascaridia*

galli can cause loss of body weight, intestinal hemorrhage, increased mortality by small intestinal obstruction (Permin *et al.*2006; Gauly *et al.*, 2007). *Heterakis gallinarum* infection usually do not show any clinical signs but it is an important transport host of blackhead, *Histomonas meleagridis* (McDougald, 2005). Cestodes (tapeworm) infected chickens also show villous atrophy, catarrhal enteritis, granuloma formation in duodenum, inflammatory reaction and vaculation of epithelial cells (Kurkure *et al.*1998).

Parasitic disease are problems wherever poultry are raised, whether in large commercial operations or in small backyard flocks, which causes a significant loss on poultry industry (Molin 1885). In most cases, presence of a few parasites does not usually cause a problem however, large numbers can have a devastating effect on growth, egg production, etc. (Tesfaheywet *et al.*2012). The chickens pick up the parasite eggs directly by ingesting contaminated feed, water litter or by eating snails, earthworms, millipedes and other insects which carry the eggs of the parasites (Butcher & Miles, 2012).

Parasitic infection in chicken is the major problem of developing countries like Nepal which leads to economic losses of farmers and ultimately to nation. Domestic fowls are more often infected due to the poor management systems, lack of veterinary services and knowledge about the parasites. Actually the parasites can't be totally eradicated but their number can be controlled so, to prevent such infection first of all, detail study of those parasites should be done including their life cycle and medication should be followed.

1.4 Objectives

1.4.1 General Objective

To determine the prevalence of gastro- intestinal parasites in Kadaknath and cross breed Kadaknath chicken in Suddodhan rural municipality, Rupandehi,Nepal.

1.4.2 Specific Objective

a. To determine the prevalence and intensity of GI parasites of Kadaknath chicken.
b. To determine the prevalence and intensity of GI parasites of Cross breed Kadaknath.
c. Comparison of GI parasites between Kadaknath and Cross breed.

1.5 Rationale of the study

Kadaknath are prone to various parasitic diseases due to their coprophagic nature which directly hampers the Kadaknath farming industry. Till date there has no any studies being carried out regarding gastro-intestinal impediments and overall status of Kadaknath in Nepal. As Kadaknath farming is new in Nepal so, it is important to know about the factors that affect them in the farming which may be abiotic and some biotic factors. One of the biotic factors is parasites. Hence, this study will help to document overall gastro-intestinal impediments to improve Kadaknath farming or Poultry farming system in Nepal. This study will provide reference for future researchers.

2. LITERATURE REVIEW

There are some published article regarding the gastro-intestinal parasites of different poultry birds but in case of gastro-intestinal parasites of Kadaknath no any articles has been published till date. So, to fulfill the lack of literature, some articles regarding gastro-intestinal parasitic infection of some poultry birds including domestic, free-ranging, broiler, cross breed, local, exotic including indigenous have been described.

2.1 Poultry parasitic disease in global context

Poultry farming is the main source of commercial production of meat and egg. But these poultry birds are infected with gastro-intestinal parasites. Globally most of the studies were conducted from African sub continent. In Kenya (Irungu *et al.*2004) and Ethiopia (Hussen *et al.*2012) cestode parasites have been reported as the most prevalent parasites among helminthes in indigenous poultry and scavenging poultry respectively.

Idika *et al.*(2016) studied 125 free range local chickens to determine the GI helminth parasites and revealed four species of cestodes(*Raillietina echinobothridia, Raillietina tetragona, Raillietina cesticillus* & *Choanotaenia infundibulum*) and two nematode species (*Ascaridia galli , Heterakis gallinarum*). Abdelgader *et al.*(2008) reported three species of nematode parasites (*Ascaridia galli, Heterakis gallinarum a*nd *Capillaria obsignata)* and eight species of cestode parasites (*Raillietina cesticillius, Raillietina echinobothridia, Raillietina tetragona, Davainea proglottina, Hymenolepis contaniana,Hymenolepis carioca, Amoebotaenia cuneata* and *Choanotaenia infundibulum*) from Jordan. Nnadi and George, 2010 reported equal prevalence of both helminthes (35.5%) and coccidian parasites (35.5%) in Nigeria from domestic chickens. Helminthes and protozoan parasitic disease were found as a major poultry disease of parasitic origin. In Ethiopia, (Beyene *et al.*2014) and Nigeria (Fatihu *et al.*1991) carried out comparative studies of gastro-intestinal helminthes in local and the exotic chickens and revealed that the local breed chickens had high infection rate than the exotic breeds. In the study carried out by Kisia *et al.*(2004) in indigenous poultry in Kenya reported nine species of helminth parasites from the intestinal tract with highest prevalence of cestode parasites i.e *Raillietina* sp. (47.53%) with least prevalence rate of *Trichostrongylus tenius*(1.04%) followed by *Syngamus trachea*(0.40%). In Ebonyi state Nigeria, Uhuo *et al.,* (2013) reported that the local chickens slaughtered in eatery centers have highest prevalence of mixed helminthic infection (86.6%) followed by single parasitic infection

(15.3%). In Southeast Cameroon, Nghonjuyi *et al.*(2014) carried out study of gastro-intestinal parasites of scavenging chickens and reported following parasites i.e *Eimeria* sp. had the highest prevalence (33.9%) followed by *Ascaridia galli*(14.3%), *Heterakis gallinarum* (9.8%), *Strongyloides* sp.(8.6%), *Capillaria s*p. (5.7%) and *Trichuris* sp (5.74%).Solomon e*t al.*,(2017) from Ethiopia reported 51.8% total prevalence out of 384 samples and revealed highest prevalence of *Ascaridia galli* (28.6%) followed by *Heterakis gallinarum* (8.6%) and *Raillietina s*p. (2.5%) and 11.5% samples were found to have mixed infection from different poultry management systems.

Luka and Ndams (2007) carried out study in GI parasites of domestic chicken in Samura, Nigeria and revealed protozoans were most prevalent followed by cestodes and nematodes and among protozoans, seven species of *Eimeria* has been recorded i.e *Eimeria tenella, Eimeria brunette, Eimeria mitis, Eimeria necatrix, Eimeria maxima, Eimeria acervulina* and *Eimeria mivati.* Similarly, Lobago *et al.*(2005) carried out study on coccidiosis from 965 dead chickens and revealed 38.34% birds were found to have clinical coccidiosis and recorded four species of *Eimeria* i.e *Eimeria brunette*(45.3%), *Eimeria tenella*(40.8%), *Eimeria acervulina* (9.7%) and *Eimeria necatrix* (4%). Shahin *et al.,* (2011) carried out study of prevalence of cestodiasis in Egypt from 860 chickens and recorded five species of cestode parasites i.e *Raillietina tetragona, Raillietina echinobothdrida, Raillietina cesticillus, Choanotaenia infundibulum and Raillietina ransomi* was first recorded from Egypt. In South western Nigeria, Agbolade *et al.*(2014) carried out study of gastro-intestinal parasites of domestic fowls comprising 110 exotic and 23 local domestic fowls and revealed 37.6% overall prevalence and the parasites recorded were *Ascaridia galli, Capillaria sp, Heterakis gallinarum, Syngamus trachea, Raillietina* sp., *Giardia* sp., *Trichostrongylus gallinae, Eimeria* sp. and *Plasmodium* sp. Hadi *et al.*(2011) carried out prevalence of *Eimeria* sp. in scavenging chicken in Iran and reported 128 chickens were infected with coccidiosis out of 200 native chickens and recorded three species of *Eimeria* sp. i.e. *Eimeria tenella* had the highest prevalent(24%) followed by *Eimeria acervulina* (18%) and *Eimeria maxima* (10%). In Oromiya region, Africa, Solomon & Yobsan (2017) studied the prevalence of gastro-intestinal helminthes in free range backyard chickens and revealed the following parasites *Ascaridia galli, Heterakis gallinarum, Raillietina echinobothridia, Raillietina tetragona, Capillaria* sp., *Syngamus trachea, Davainea proglottina, Raillietina cesticillus, Choanotaenia infundibulum* and *Amoebotaenia cuneata.* In Ethiopia, Belihu *et al.*(2011) reported five

species of nematodes i.e. *Ascaridia galli, Heterakis gallinarum, Capillaria* sp., *Trichostrongylus tenius* and *Subulura* sp. and seven species of cestodes i.e. *Raillietina tetragona, Raillietina echinobothridia, Raillietina cesticillus, Davainea proglottina, Amoebotaenia sphrnoides, Choanotania infundibulum* and *Hymenolepis* sp. from local chickens.

In Europian continent, Kurt and Acini (2008) reported altogether sixteen species of helminthes parasites from scavenging chicken with the prevalence rate 88% followed by layers from laying battries 4% but none broiler harboured helminthes from Turkey. Among helminth species *Ascaridia galli* (Permin *et al.*1999; Wuthijaree *et al.*2017; Sherwin *et al.*2013; Kurt& Acini, 2008) , *Capillaria s*p.(Baboolal *et al.*2012; Permin *et al.*1999;Wuthijaree *et al.*2017;) *Raillietina s*p. (Shahin *et al.* 2011; Permin *et al.*1999), *Syngamus trachea* (Sherwin *et al.*2013; Kurt&Acici, 2008) have been stated as common helminth parasites among poultry birds. Jansson *et al.*(2010) studied ascarid infections in laying hens kept in different housing systems and revealed Ascarid infection rare in caged flocks and more in non-caged systems. In France, Williams *et al.*(1996) carried out a survey in commercially reared chickens and revealed six species of *Eimeria* sp infection i.e. *Eimeria acervulina*(100%), *Eimeria mitis* (82%), *Eimeria tenella* (77%), *Eimeria maxima*(73%), *Eimeria praecox*(45%) and *Eimeria brunetti* (27%).

In India, Kumar *et al.*(2015) conducted gastro-intestinal parasitic infections in chicken in 58 poultry farms, 81.03% were positive for *Eimeria* sp. 15.52% *Ascaridia galli,* 3.45% *Heterakis gallinarum,* 1.72% *Trichostrongylus tenuis.* Among helminth, nematode species is more common and reported species includes: *Ascaridia s*p., *Heterakis* sp., and among trematode: *Raillietina s*p. (Alam *et al.*2014; Naphade, 2013; Satish & Mishra, 2013; Sahu & Sinha, 2016). Among protozoan, *Eimeria s*p. is more common and reported species are: *Eimeria tenella, Eimeria maxima, Eimeria acervulina, Eimeria necatrix* (Ayaz *et al.*2003; Awais *et al.*2012; Nematollahi *et al.*2001; Gharekhani *et al.2*014). In Kashmir, India Dar *et al.*(2013) revealed nine species of cestodes i.e *Raillietina tetragona, Raillietina cesticillus, Raillietina echinobothridia, Raillietina spiralis, Choanotaenia infundibulum, Choanotaenia gondwana, Amoebotaenia cuneata, Amoebotaenia domesticus* and *Davainea proglottina* with highest prevalence of *Raillietina tetragona.* In Bangladesh, (Rabbi *et al.2*006) studied 240 viscera from broiler, layer and backyard chickens and revealed 100% helminth infection in backyard chickens followed by layer(48.75%) and broiler (3.75%). In China, Wang *et al.*(2014) studied a

total of 385 broiler chicken 38(10%) samples were positive for *Cryptosporidium* infection and recorded three species i.e. *Cryptosporidium baileyi, Cryptosporidum meleagridis* and avian genotype II. In Pakistan, Bachaya *et al.*(2012) studied detection of different *Eimeria* sp. causing coccidiosis in layer chickens and revealed four different *Eimeria* sp. i.e *Eimeria tenella* had the highest prevalence rate (39.93%) followed by *Eimeria maxima* (30.20%), *Eimeria mitis* (19.13%) and *Eimeria necatrix* (10.74%) and among them young layer chickens had the highest prevalence(60.16%) over adult chickens(37%).

2.2 Poultry parasitic disease in national context

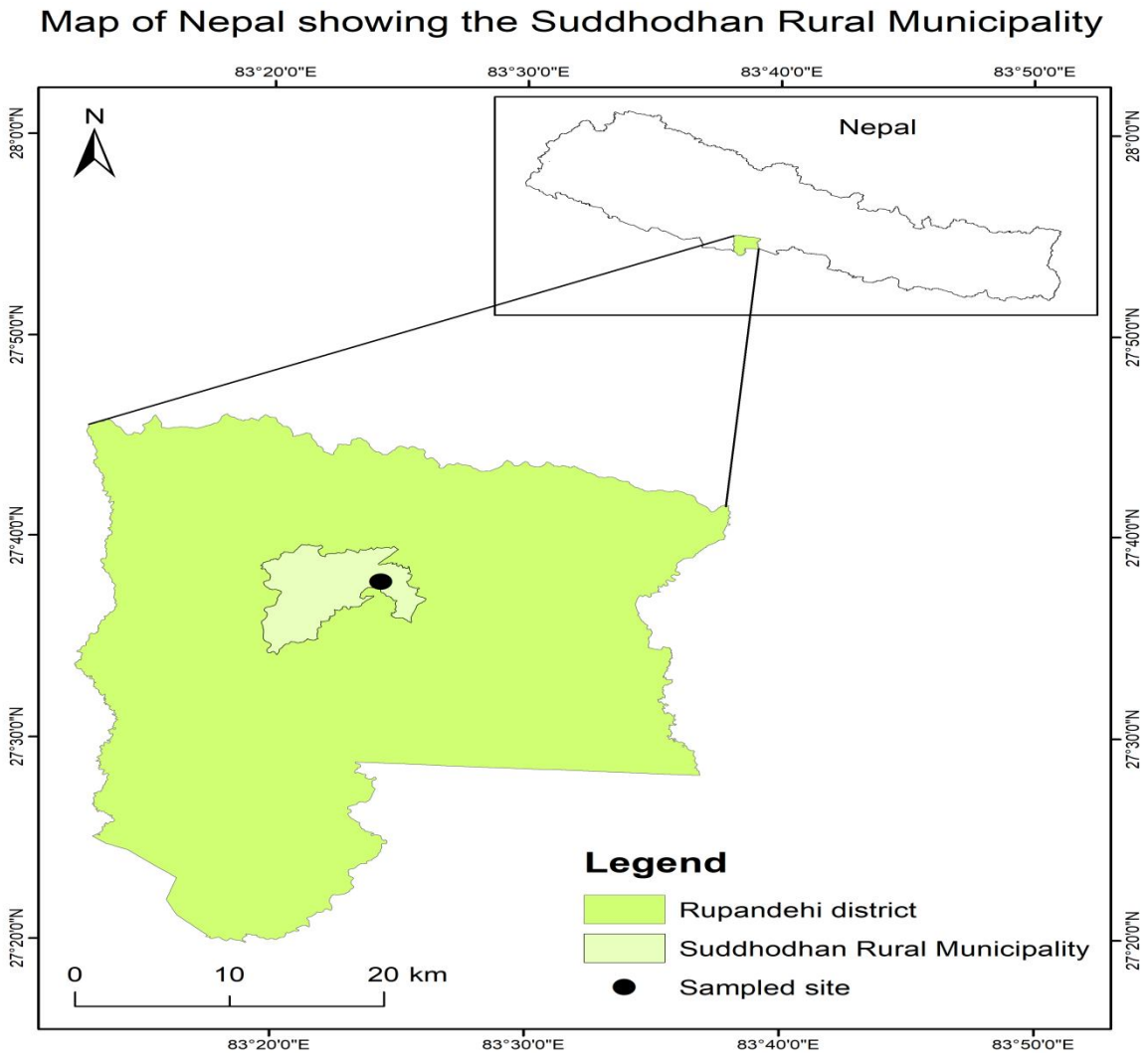
In the context of Nepal, no any article regarding gastro-intestinal parasites of Kadaknath is found. However, some articles regarding GI parasites of poultry birds have been described.

In Lalitpur, Subedi *et al.*(2018) reported five species of nematodes of chickens from Lalitpur i.e *Heterakis gallinarum* (22.4%), *Capillaria* sp.(16%), *Ascaridia galli*(10.4%), Unidentified (4.8%) and *Raillietina teragona*(4%). Similar helminthes parasites reported are: *Ascaridia galli* (Rai, 1988; Shrestha,1990), *Heterakis gallinarum* (Rai,1988;Shrestha, 1990), *Raillietina cesticellus, Raillietina tetragona, Raillietina echinobothridia*(Rai, 1988), *Amoebotaenia cuneata, Contugnia digonopora* (Shrestha, 1990), *Fernandezia kantipuri, Catatropis verrucosa, Echinostoma revolutum,* (Rai, 1988; Shrestha, 1990), *Prosthogonimus* (Rai, 1998). In Chitwan, Adhikari *et al.*(2008) identified five *Eimeria* sp. in layer chicken with highest prevalence of mixed infection (*Eimeria acervulina, Eimeria maxima, Eimeria necatrix, Eimeria brunette, Eimeria tenella).* Similar studies carried out in poultry farms of Kathmandu and Lalitpur district (Jayswal *et al.*2014) reported four species of *Eimeria* with the highest prevalence rate of *Eimeria tenella.*

3. MATERIALS AND METHODS

3.1 Study area

Suddodhan rural municipality is located within Rupandehi District. Rupandehi district is situated in 83°22'44.18"E longitude and 27°37'35.22"N latitude at 100-300m of elevation. It covers an area of 1360 sq.km. Kadaknath farm is located in Belawa near to the Lumbini farmland 15 km north to the birth place of Lord Buddha. The study area is area of 12 bigha.



3.2 Materials

Following materials have been used during the research work:

i. Electric microscope

vii. Centrifuge machine

ii. Collecting vials

iii. Gloves

iv. Glass slides

v. Cover slips

vi. Stage micrometer and ocular micrometer

vii. Centrifuge tube

ix. Measuring cylinders

Chemicals Requirements

Potassium dichromate, normal saline, distilled water, lugol's iodine solution, methylene blue and saturated sodium chloride (NaCl).

3.3 Methods

3.3.1 Sample size

To study the gastro-intestinal parasites of Kadaknath a total of 150 samples were collected comprising 100 of Kadaknath and 50 of cross breed Kadaknath from the farm (Photo 1) in Suddodhan rural municipality, Belawa, Rupandehi.

3.3.2 Sample collection and preservation

About 20-25 gm of fecal sample was collected with the help of caretaker from different place of the farm in morning time. The collected fecal sample were placed in sterile vial then filled with 2.5% potassium dichromate to cover the fecal sample completely. All the samples collected were labeled properly. After that, vials were kept in air tight cool box. Than the sample were brought to Central Department of Zoology.

3.3.3 Laboratory examination

After preservation and transportation of fecal samples, examination of all sample were processed at the laboratory of Central Department of Zoology, T.U. Kirtipur, Kathmandu. The fecal samples were examined under microscope (photo 2) for trophozoite, cysts, oocysts, eggs and larvae of gastro-intestinal parasites by stained smear preparation and concentration method viz. floatation and sedimentation techniques (Soulsby, 1982). The Stoll's count technique was used to determine mix infection and intensity of parasites (Soulsby, 1982).

3.3.3.1 Stained smear preparation

Preparation of smear is required for many laboratory procedures. The purpose of making smear is to fix the parasitic cysts/ova/eggs onto the slide. It is useful to study the nuclear character and identification of protozoan cysts. A small portion of fecal sample was picked up with a clean bamboo toothpick and emulsified with Lugol's iodine solution on a clean glass slide and covered with a cover slip. The smear was examined under electric microscope at 10X and 40X.(Soulsby,1982)

3.3.3.2 Concentration methods

The concentration procedures include floatation and sedimentation techniques for the detection of eggs/cysts/trophozoites/larva of parasites (Soulsby, 1982). In case of heavy infection, parasites can be easily seen in smears but in case of light infection it is difficult to detect the parasitic form in smears or mounts. Hence, in the study, concentration methods (Floatation and Sedimentation) were carried out.

a. Floatation Techniques

About 1 gm of feces was taken in a glass pestle and 10ml of distilled water was added and mixed well. Suspension was strained to remove the debris. The suspension was centrifuged at 1000 revolution per minute (rpm) for 5 minutes (photo 3). The tube's water was replaced with super saturated NaCl solution and again centrifused. After centrifused, more saturated NaCl solution was added to develop convex meniscus at the top of the tube and one drop of Methylene blue was also added (photo 4). The eggs and cysts float to the top and were collected by placing a cover slip on the surface of the meniscus at the top of the tube. The surface layer was examined under microscope (Soulsby, 1982)

b. Sedimentation Technique

About 1gm of feces was taken in a glass pestle and 10ml of distilled water was added to it and mixed well. Suspension was strained to remove the debris and poured into a centrifuge tube up to an inch below the brim. Centrifuged at 1000 revolutions per minute (rpm) for 5 minutes. The supernatant was discarded and form the sediment. Iodine wet mount was prepared for each samples by mixing 1-2 drops of the sediments with Lugol's iodine solution in a glass slide and examined under microscope by covering with a cover slip.(Soulsby, 1982)

3.3.4 Identification

Prepared slides were examined under microscope under 10X and 40X respectively. Size of eggs/oocysts/cysts were measured using stage and ocular micrometer. The eggs, cysts and larva were identified by comparing the structure, color and size of eggs, cysts and larva of published articles, journals and books (Castonon, *et al.*2007; Belete, 2016; Rahimian, 2016)

3.4 Data analysis

The study was focused on identification of different intestinal parasites, the data were analyzed by using MS-Excel 2007 and statistical analysis was performed using "R", version 3.5.2 software package with chi-square test. In all cases 95% confidence interval (CI) and $P<0.05$ was considered for statistically significant association.

Photo 1: Kadaknath chicken in farm

Photo 2: Microscopic observation of samples

Photo 3: Centrifuging the sample

Photo 4: Flotation technique

4. RESULTS

Altogether 150 fresh fecal samples (Kadaknath-100 and Cross breed of Kadaknath- 50) were collected to determine the prevalence of gastro-intestinal parasites. As, Kadaknath chickens were raised in a farm, the fresh fecal samples were collected from the farm.

4.1 Prevalence of parasitic infection in Kadaknath (*Gallus gallus domesticus)*

Kadaknath chickens were found to be infected with overall 54% parasitic prevalence. These chickens were found to be infected with two species of protozoan parasites and four species of helminthes parasites.

About 19% of samples collected from Kadaknath were found to be positive for *Eimeria* sp. They were infected by two species of *Eimeria* i.e *Eimeria tenella* and *Eimeria maxima*. They were categorized on the basis of their structure and size. Infection rate with *Eimeria tenella* (12%) was found comparatively higher than *Eimeria maxima* (7%) in Kadaknath chicken (Table 1).

Only two groups of helminthes i.e nematodes and cestodes were observed in Kadaknath samples. Kadaknath were found to be infected with three species of nematodes and one species of cestodes parasites. Prevalence of nematode were observed comparatively higher than that of cestodes i.e 42% and 4% respectively during the study. Among nematode parasites, *Ascaridia* sp. belonging to roundworm family were found to have high prevalence than that of *Heterakis* sp. Likewise, *Trichostrongylus* sp. was found to be least prevalent in Kadaknath with 2%. Among cestode parasite only one species was recorded i.e. *Raillietina* sp. with prevalence of 4% (Table 1).

Table 1: Gastro-intestinal parasites of Kadaknath chicken

Class	Parasites	Prevalence (N=100)	Photo No.
Sporozoa	*Eimeria tenella*	12%	5
	Eimeria maxima	7%	6
	Total	**19%**	
Nematode	*Ascaridia* sp.	35%	7
	Heterakis sp.	12%	8
	Trichostrongylus sp.	2%	9
	Total	**42%**	
Cestode	*Raillietina* sp.	4%	10

Concurrency of parasitic infection in Kadaknath revealed single infection (70.37%)was found prevalent over multiple infection and within single infection, *Ascaridia* sp infection was found highest (60.52%) followed by *Eimeria maxima* (13.15%) whereas in double parasitic infection *Heterakis* sp. & *Eimeria tenella* occurred at the rate of 85%. Triple infection occurred in 3.7% of the samples, in which maximum parasitic combination revealed *Ascaridia s*p, *Eimeria tenella* and *Heterakis* sp. (Fig.1)

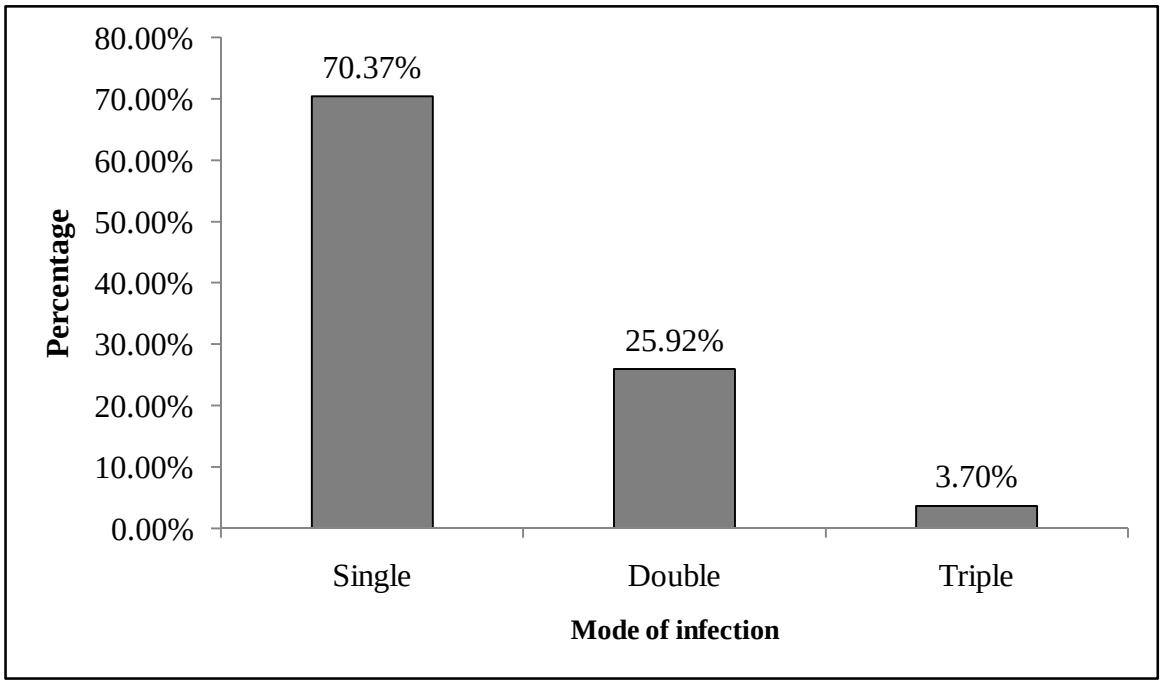


Figure 1: Concurrency of parasitic infection in Kadaknath

Most of the Kadaknath were found infected with light parasitic infection (23%) which are often asymptomatic or non- pathogenic in Kadaknath whereas 10% of chicken were infected by *Ascaridia* sp. with mild infection. Only two species of parasites showed heavy infection i.e *Eimeria tenella* and *Ascaridia* sp. (Table 2)

Table 2: Intensity of GI parasites of Kadaknath

	Name of parasites	**light (+)**	**mild (++)**	**moderate (+++)**	**heavy (++++)**
Sporozoa	*Eimeria tenella*	3	2	2	5
	Eimeria maxima	4	1	2	-
Nematode	*Ascaridia* sp.	5	10	5	15
	Heterakis sp.	5	7	-	-
	Trichostrongylus sp.	2	-	-	-
Cestode	*Raillietina* sp.	4	-	-	-

Note: Light infection= <2 eggs/oocysts/larvae per field

Mild infection= 3-4 eggs/oocysts/larvae per field

Moderate infection= 5-6 eggs/oocysts/larvae per field

Heavy infection= > 6 eggs/oocysts/larvae per field

4.2 Prevalence of GI parasites of cross breed Kadaknath

A total of 50 fresh fecal samples of cross breed Kadaknath were collected from the farm. Cross breed were found to be infected with 44% parasitic prevalence. Furthermore, cross breed were found to be infected with two species of protozoan parasites and three species of helminthes parasites.

About 34% of samples collected from cross breed Kadaknath were found to be positive for *Eimeria* sp. They were infected with two species of *Eimeria* i.e. *Eimeria tenella and Eimeria maxima.* Prevalence of *Eimeria maxima* was higher i.e 18% as compared to *Eimeria tenella.*(Table 3)

Only one group of helminthes i.e. nematodes were observed in cross breed samples. Cross breed were found to be infected with three species of nematodes. Among nematode parasites, *Ascaridia s*p. belonging to roundworm family were found to have high prevalence than that of *Heterakis* sp. Likewise, *Trichostrongylus* sp. belonging to

hookworm family was found to have least prevalent in Cross breed with 10%. No any cestode and trematode were observed in Cross breed of Kadaknath. (Table 3)

Table 3: Prevalence of GI parasites of Cross breed Kadaknath

Class	Parasites	Prevalence (N=50)	Photo No.
Sporozoa	*Eimeria tenella*	16%	11
	Eimeria maxima	18%	12
	Total	**34%**	
Nematode	*Ascaridia* sp.	24%	13
	Heterakis sp.	20%	14
	Trichostrongylus sp.	10%	15
	Total	**38%**	

Concurrency of parasitic infection in Cross breed Kadaknath revealed double infection (50%) was found maximum as compared to single and multiple infection. Combination of *Ascaridia* sp. and *Hetreakis* sp. had the highest prevalnce of double infection i.e 81.81%. Whereas, single infection was found 27.27% among cross breed chicken and within single infection *Ascaridia* sp. had the highest (80%) prevalence rate followed by *Eimeria maxima* i.e. 10% (Fig 2)

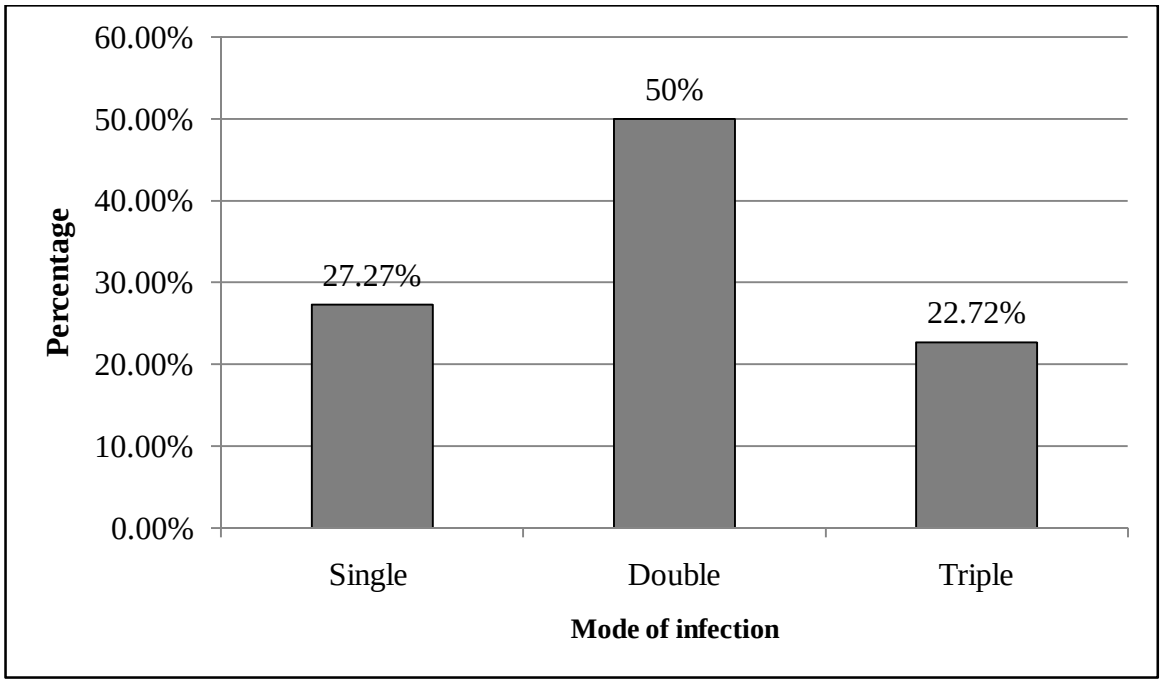


Figure 2: Concurrency of parasitic infection in cross breed Kadaknath

Most of the parasites of Cross breed revealed light infection with 46% and among them *Heterakis* sp. had the highest prevalence of light infection while almost four species of parasites revealed mild rate of intensity i.e. 24% and three species of parasites revealed moderate rate of intensity i.e. 12%. While only one species of parasites i.e. *Eimeria maxima* were found to prevailed with heavy infection i.e. 4% which indicates high prevalence of *Eimeria maxima* in Cross breed Kadaknath. (Table 4)

Table 4: Intensity of GI parasites of Cross breed Kadaknath

Class	**Name of parasite**	**Light (+)**	**Mild (++)**	**Moderate (+++)**	**Heavy (++++)**
Sporozoa	*Eimeria tenella*	3	3	2	-
	Eimeria maxima	4	-	3	2
Nematode	*Heterakis* sp.	7	3	2	-
	Ascaridia sp.	5	5	-	-
	Trichostrongylus sp.	4	1		

4.3 Comparision of overall prevalence of Kadaknath and cross breed Kadaknath

Among 100 samples collected from Kadaknath only 54% samples were positive for overall gastro-intestinal parasites and among 50 samples collected from Cross breed of Kadaknath 44% samples were found to be positive for overall GI parasites which indicates Kadaknath were more susceptible to GI parasites in comparison to Cross breed Kadaknath.

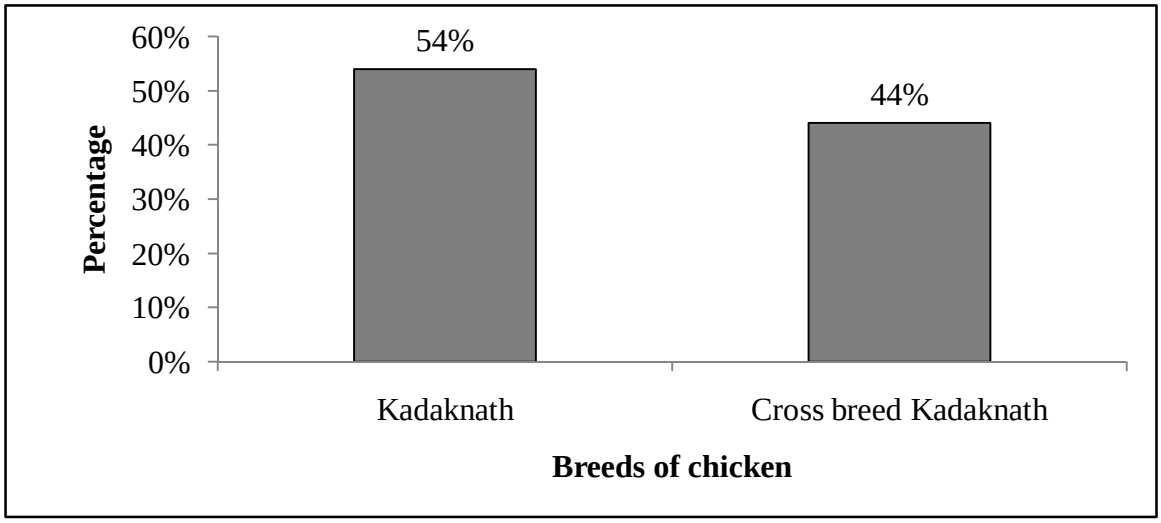


Figure 3: Comparison of overall prevalence of GI parasites between Kadaknath and Cross breed Kadaknath

Kadaknath and Cross breed of Kadaknath were found to have *Eimeria tenella and Eimeria maxima* as common protozoan parasites. The prevalence rate of *Eimeria tenella* in Kadaknath were found to be almost similar to that of Cross breed Kadaknath i.e 12% and 16% respectively. Statistically, there was no significant difference with regard to *Eimeria tenella* of Kadaknath and cross breed Kadaknath (P=0.671) and the prevalence rate of *Eimeria maxima* in Kadaknath were found to be less than the rate of Cross breed Kadaknath i.e. 7% and 18% respectively. Although statistically, there is no significant difference observed in Kadaknath and Cross breed Kadaknath (P=0.075) indicating that both pure breed Kadaknath and Cross breed Kadaknath are equally susceptible for both *Eimeria* sp. (Table 5)

Both groups of chicken were found to be infected with three species of nematode parasites i.e *Ascaridia* sp, *Heterakis* sp and *Trichostrongylus* sp. Among them, only *Trichostrongylus* sp infection was found significantly high in cross breed chicken compared to pure breed of Kadaknath (P=0.033). While both groups of chicken were found almost equally infected with *Ascaridia* sp. and *Heterakis* sp. without any significant difference (Table 5)

Table 5: Comparison in GI parasites between Kadaknath and Cross breed Kadaknath

Class	**Parasites**	**$\chi 2$ value**	**P-value**
Sporozoa	*Eimeria tenella*	0.180	0.671
	Eimeria maxima	3.15	0.075
Nematode	*Ascaridia* sp.	2.89	0.088
	Heterakis sp.	2.73	0.098
	Trichostrongylus sp.	4.79	0.033
Cestode	-	-	-

4.4 Comparison of concurrency of parasites

Both groups of chicken were found to have single, double and triple mode of infection. Statistically, there was no significant difference (P=0.078) between double mode of

infection indicating that both chickens were equally susceptible to double mode of infection and single and triple mode of infection were found to be statistically significant between these chickens. As in pure breed of Kadaknath,infection of *Ascaridia* sp was significantly high and in triple mode of infection cross breed of Kadaknath had higher infection rate. Significantly higher number of cross breed were found to be infected with not a single parasite but combination of *Ascaridia sp, Heterakis sp* and *Eimeria tenella.*

Table 6: Comparison of concurrency of parasites between Kadaknath and Cross breed Kadaknath

Mode of infection	**$\chi 2$ value**	**P-value**
Single	10.20	0.001
Double	3.086	0.078
Triple	6.76	0.021

4.5 Photos of Eggs/Oocysts/ of GI Parasites of Kadaknath (10X40X)

Photo 5: Oocyst of *Eimeria tenella*(27.5µm/25µm)

Photo 6:Oocyst of *Eimeria maxima*(32.5µm/35µm)

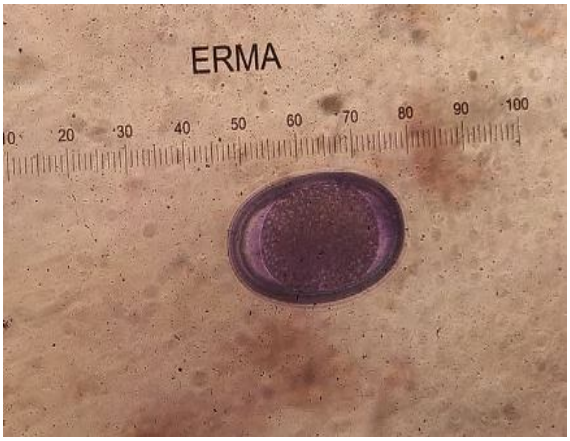


Photo 7: Embryonated egg of *Ascaridia* sp.(77.5µm/47.5µm)

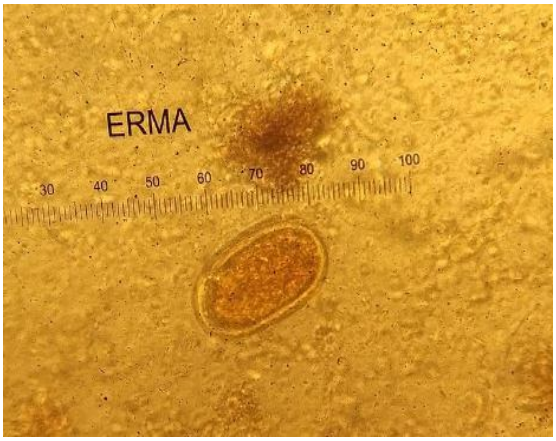


Photo 8: Egg of *Heterakis* sp. (70µm/55µm)

Photo 9: Egg of *Trichostrongylus* sp. (77.5µm/ 52.5µm)

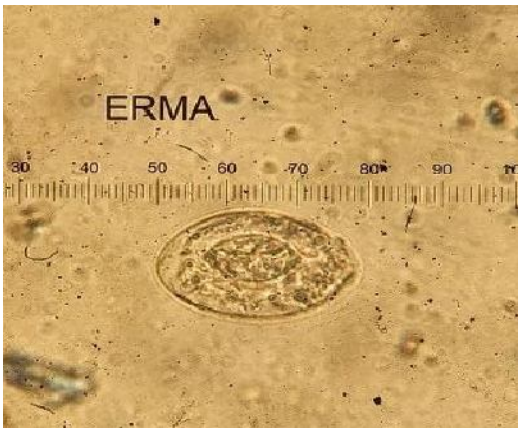


Photo 10: Egg of *Raillietina* sp.(97.5µm/47.5µm)

4.6 Photos of eggs/ oocyst of GI parasites of Cross breed (10X40X)

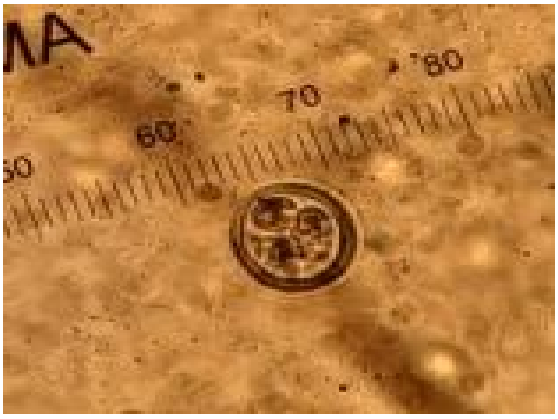


Photo 11: Oocyst of *Eimeria tenella* (25µm/22.5µm)

Photo 12: Oocyst of *Eimeria maxima* (30µm/25µm)

Photo 13: Egg of *Ascaridia* sp. (75µm/45µm)

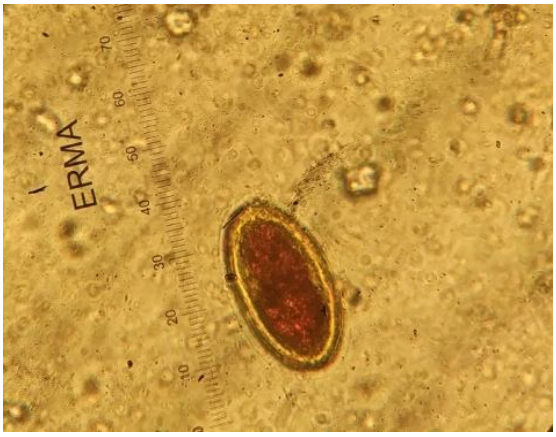


Photo 14: Egg of *Heterakis* sp. (80µm/37.5µm)

Photo 15: Egg of *Trichostrongylus* sp.(77.5µm/60µm)

5. DISCUSSION

Poultry farming is one of the most emerging agriculture industry. Poultry farming has been done in commercial scale for the daily fulfillment of meat and egg for the people. Poultry farming includes raising of broiler chickens but with the change in taste of people other fowls like turkey, duck,local breeds, Kadaknath etc have also been now introduced in poultry farming. Kadaknath farming has been first started in India and recently it has also been raised in farm of Farsatikar, Rupandehi, Nepal. Poultry farming also needs some attention as most of the farmers are unknown about the disease of poultry. There are some viral and bacterial disease like bird flu, Newcastle disease (Alamargot *et al.*1985), Avian pox, chronic respiratory disease (Alamargot *et al.*1985), coliform infection (Glisson, 1998) etc. that causes high mortality and morbidity leading to large economic loss to the farmers.

Besides viral and bacterial disease, parasitic diseases are often neglected in poultry industry. Among parasites, most of the protozoan parasites such as coccidian parasites can cause heavy loss due to high mortality and morbidity (Morris *et al.*2007). Similarly the helminth parasites can also cause directly and indirectly heavy loss in poultry farming because it retards the growth of the chickens (Ashenafi & Eshetu, 2004)

Since Kadaknath farming is new in context to Nepal, this is the first study related to the GI parasites of Kadaknath chicken. A total of 54% Kadaknath chicken were found infected with parasitic disease. In the present study 19% fecal samples were found to be positive for protozoan parasites in Kadaknath chicken. Prevalence rate found in Kadaknath was found to be lower compared to other reports on broiler chickens from Bangladesh (Iqbal and Begum 2008), Turkey (Karaer *et al*.2012) , Pakistan(Ayaz *et al.*2003; Awais *et al*.2012) and Quraishy *et al*.(2009) in Riyadh city, Saudi Arabia. The finding is somewhat similar to the finding of Adhikari *et al.*(2008) among layer chickens in Ratnanagar municipality, Chitwan. In the broiler chicks, nine *Eimeria* sp. had been reported and among them *E. brunetti, E. maxima, E. necatrix, & E. tenella* were highly pathogenic, *E. acervulina, E. mitis, E. mivati* were rather less pathogenic and *E. praecox & E. hagoni* were regarded as the least pathogenic (Thebo *et al.*1998; Al-Natour & Suleiman, 2002). But in Kadaknath chickens of this study reports two species of *Eimeria* i.e. *Eimeria tenella* and *Eimeria maxima*. They have a single host in which they undergo asexual (schizogony, merogony) and sexual (gametogony) multiplication. Various species of *Eimeria* have been recorded by Nematollahi *et al*.(2009) in Tabriz, Iran i.e. *Eimeria*

acervulina, *Eimeria tenella*, *Eimeria necatrix* ,*Eimeria maxima* and *Eimeria mitis* with highest prevalence of *E. acervulina* followed by *E. tenella* (14.22%) and *E. maxima* (5.5%)which is somewhat similar to the present finding. Seven species of *Eimeria* have been identified by Ayaz *et al.*(2003) with highest prevalence of *Eimeria tenella* (50%) followed by *Eimeria maxima* (40%) which is higher as compared to the present finding. Adhikari *et al.*(2008) reported five different species of *Eimeria*, among them *Eimeria tenella* had the highest prevalence (25%) and *Eimeria maxima* as the least prevalence (5%).

Prevalence of helminth infection (45%) was found higher than the protozoan parasitic infection. This finding was also supported by Puttalakshmamma *et al.*(2008) who revealed higher prevalence of helminth infection (71%) than protozoan (10%) from 100 desi and 100 farm birds in and around Banglore. Among helminthes two groups of helminthes i.e. nematodes and cestodes were recorded from Kadaknath pure breed. Similar finding has been reported by Naphade (2013) from broiler poultry birds of India, similarly Baboolal *et al.*(2012) from Trinidad, South Carribean and Tesfaheywet *et al.*, (2012) from Ethiopia had also reported similar results. Prevalence of nematode were observed comparatively higher than that of cestodes i.e. 42% and 4% respectively in present study. The higher prevalence of nematodes over cestodes has also been reported in commercial layers of Pakistan (Sayeed *et al.*2000), poultry birds of India (Naphade, 2013) and from Ethiopia (Tesfaheywet *et al.*2012). Similar, comparatively high prevalence rate of *Ascaridia* sp. over *Heterakis* sp. has been reported from broilers chicken of Pakistan (Sayed *et al.*2000), Palestine (Rayyan & Al-Hindi 2010). This result strongly suggested that *Ascaridia* sp. is the commonest and most important helminth infection of poultry. Infestation with *Ascaridia galli* causes reduction in the growth rate and weight loss, which may be related to damage to the intestinal mucosa. *Ascaridia galli* significantly affects the health of chickens by sharing the feed consumed by the host thus causing stunted growth and reduced egg and meat production (Eshetu *et al.*2001, Ashenafi & Eshetu 2004). Although this and other studies showed that *Heterakis gallinarum* was recorded with a relatively lower prevalence compared to with *Ascaridia galli* , its pathology and its role as a carrier of an important pathogen namely, *Histomonas meleagridis* in turkeys and chickens(Ashenafi& Eshetu, 2004) should be highly regarded. In the present study *Trichostrongylus* sp. belonging to nematode class has been recorded with the lower prevalence rate i.e. 2%. The finding is somewhat similar to the finding

reported by Kumar *et al.*(2015) in Uttarakhand and Uttar Pradesh. In the present study only one species of cestode i.e *Raillietina* sp. was recorded with the prevalence of 4%. Infection *with Raillietina* sp in broiler chicken with lower prevalence has also been reported from India (Naphade, 2013), from Ethiopia (Tesfaheywet *et al.*2012) and Makurdi Township (Ogbaje *et al.*2012). The prevalence rate was somewhat similar to the finding of Fatihu *et al.,* (1991) who reported only *Raillietina tetragona* among the exotic chickens. Three species of cestodes had been reported by Baboolal *et al.*(2012) i.e. *Raillietina echinobothrida, R. cesticillus* and *Choanotaenia infundibulum* from broiler chickens in Trinidad, South Carribean. This could be due to difference in the season of conducting these studies, availability of intermediate hosts, individual host resistance and ecological parameters. The presence of only *Raillietina* sp. could also be due to the possible different management practices applied in the farms. Parasitic cestodes in poultry are known to cause retarded growth, enteritis, diarrhea and hemorrhages even in severe cases it may cause death of young birds. Thus the 4% in the present finding should not be undermined.

There were no trematodes reported from the present study. This finding is supported by Baboolal *et al.*(2012) and Naphade(2013) from India. The absence of these parasites appeared to be linked with the complex life cycle requiring atleast an intermediate host which is aquatic. This helps to break life cycle where water is not available and hence reducing the spread of parasites. In the present study, the single parasitic infection was found to be more common in Kadaknath chicken. This finding is similar to the finding reported by Ogbaje *et al.*(2012) i.e 37.5% had single infection followed by double infection(23.9%) and 13.6% had the triple infection.

Cross breed Kadaknath are the cross of local chickens and Kadaknath. In the present study the overall prevalence of gastro-intestinal infection was 44%. The finding was lower than the finding reported by Luka & Ndams (2007) from Nigeria and Nnadi & George (2010) from South Eastern Nigeria while the present finding was higher than the finding reported by Afolabi *et al.*(2016) from Akure, Nigeria.

In the present study among the cross breed chicken 34% of samples were positive for coccidian parasites. Similar prevalence rate (33.3%, 35.5%, 31.8%) has been reported from Zaria (Jatau *et al.*2012), from Nigeria (Nandi & George *et al.*2010) and from Nigeria (lawal *et al.*2016) respectively. The present prevalence rate was found higher as compared to the findings of Hamid *et al.*(2018) and Oljira *et al.*(2012) from Ethiopia.

But the present prevalence rate is lower as compared to the finding of Adang *et al.* (2016) from Nigeria. Among coccidian parasite only two species have been recorded in this study i.e *Eimeria tenella* and *Eimeria maxima.* Luka &Ndams (2007) reported seven species of protozoa i.e *Eimeria tenella,Eimeria acervulina, Eimeria brunetti, Eimeria mitis, Eimeria necatrix, E.mivati and E. maxima* with highest prevalence of *E. tenella a*nd least prevalence of *E. mivati* but the result of present finding has almost equal prevalence of both species of *Eimeria* in cross breed Kadaknath. Hamid *et al.*(2018) reported highest prevalence of *Eimeria tenella* followed by *Eimeria maxima* but the present finding has a little bit higher prevalence of *E. maxima* compared to *E. tenella* without statistical significant difference. The present prevalence rate of *E. tenella* is lower as compared to finding reported by Hamid *et al.*(2018) from Indonesia and by Adang *et al.*(2016) in Gombe State, Nigeria. The infection of *Eimeria maxima* in the present study was somewhat similar to the finding by Adang *et al.* (2016) and higher as compared to Jayswal *et al.*(2014) from poultry farms of Kathmandu and Lalitpur districts.

The parasitic nematodes that infect animals cause serious diseases that are deleterious to human health and agricultural productivity. The present study revealed 38% of nematode infection. Similar finding has been reported by Nnadi & George (2010) but present prevalence rate is lower (75.79%) as compared to reports of Ashenafi *et al* (2004) from Ethiopia. Among nematodes *Ascaridia* sp. had the highest prevalence in cross breed as in pure breed. Similar Ascariasis prevalence had been shown from various studies in cross breed local chicken (Afolabi *et al*.2016; Kaingu *et al*.2010; Uhuo *et al.*2013 and Ashenafi *et al*.2004). As in pure breed Kadaknath chicken, in cross breed chicken also prevalence rate of *Ascaridia* sp. was found comparatively higher than *Heterakis* sp. Similar nematode parasitic prevalence had been documented by other researchers from different countries from cross breed local chickens (Luka & Ndams 2007); Uhuo *et al.* 2013 and Afolabi *et al.*2016). In the present study, only three species of helminthes and one species of protozoa have been reported. But some other researchers reported three types of protozoa (*Eimeria s*p, *Histomonas meleagridis* & *Giardia lamblia)* and five types of nematodes (*Ascaridia galli, Heterakis gallinarum, Capillaria* sp., *Syngamus trachea, Trichostrongylus tenius)* by Afolabi *et al.*(2016). Ashenafi *et al.*(2004) reported six species of nematodes and cestodes , among cestodes *Raillietina echinobothridia* had the highest prevalence over *Hymenolepis containana* .Uhuo *et al.*(2013) reported two species of helminthes (*Ascaridia galli & Heterakis gallinarum),* 2 species of cestodes (*Raillietina*

tetragona & Choanotaenia infundibulum) and 1 species of trematodes (*Prosthogonimus* sp.). *Trichostrongylus* sp. had also been reported from cross breed local chicken (Agnieszka *et al.*2014; Afolabi *et al.*2016). This species was also found in cross breed Kadaknath chicken in present study. In the present study, mixed parasitic infection were found to be more common compared to single parasitic infection. This finding was supported by Uhuo *et al.*(2013),Berhe *et al.*(2019) who reported 86.6% mixed infection and 87.7% mixed infection of helminthes parasites respectively.

In comparison, the present study revealed higher prevalence of GI parasites in Kadaknath compared to cross breed. But the studies reported by Fatihu *et al.* (1991) and Beyene *et al.* (2014) revealed local chickens had higher prevalence of GI helminthes (95.7%) & (51.47%) than exotic chickens (11%) & (36.6%) respectively. Kadaknath pure breed had been introduced in Nepal has been more than two years and better adapted to the climatic conditions while cross breed Kadaknath are recently introduced. Due to the variation of exposure to the local environmental condition particularly in relation to feed and drink, Kadaknath might be more susceptible over cross breeds. Immunological factor also may play role in difference in parasitic infection in between these two group of chicken in Butwal.

Presently only one species of protozoan parasites and three species of helminthes parasites infect pure breed of Kadaknath and cross breed Kadaknath, and one species of cestode infect pure breed, other species of helminthes and protozoan parasite may also infect in near future if they are not kept under proper management systems or not given anticoccidial and antihelminthic drugs. These parasites can cause high mortality and morbidity in the chickens along with retarded growth and decrease in production of egg.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

A study was carried out to determine the prevalence of gastrointestinal parasites of pure breed of Kadaknath and cross breed of Kadaknath. A total of 150 fecal samples, Kadaknath(100) and cross breed(50) were collected from the Suddodhan rural municipality,Belawa, Rupandehi. The collected samples were examined in the laboratory for detection of GI parasites.

Among 100 fecal samples of Kadaknath, 54% samples were found to be infected with GI parasites, while among cross breed 44% samples were found to be infected. In the present study, two species of *Eimeria*, three species of nematodes and one species of cestode were recorded.

In Kadaknath, highest prevalence rate was found with *Ascaridia* sp. (35%) followed by *Eimeria* sp. (19%), *Heterakis s*p. (12%), *Raillietina s*p. (4%) and *Trichostrongylus* sp.(2%). Single mode of infection was high revealing that maximum number of pure breed Kadaknath were infected with single parasites. Most of the Kadaknath were found infected with light parasitic infection (23%) and only two species of parasites showed heavy infection i.e *Eimeria tenella* and *Ascaridia s*p.

Similary, in cross breed highest prevalence rate was found with *Ascaridia* sp. (24%) followed by *Heterakis* sp. (20%), Eimeria *maxima*(18%), *Eimeria tenella*(16%) and *Trichostrongylus* sp.(10%). Double mode of infection was high in cross breed indicating maximum number of cross breed had double parasitic infection. Most of the parasites of Cross breed revealed light infection with 46% and only one species of parasites i.e *Eimeria maxima* showed heavy infection. The study revealed that Kadaknath had higher parasitic infection compared to cross breed.

Overall comparison of parasites between both breeds showed higher prevalence in Kadaknath compared to cross breed and only *Trichostrongylus* sp. nematode parasite was found significantly high infection in cross breed compared to Kadaknath ($\chi 2=4.79$, $p<0.05$, $\alpha=1$) whereas comparison of mode of infection between two breeds revealed that there was no significant difference between double mode of infection and both single and triple mode of infection showed significant difference concluding that in Kadaknath single *Ascaridia* sp. infection was high.

6.2 Recommendations

Based on the finding of the present study, following recommendations have been drawn to reduce the risk of gastro-intestinal parasitic infection of Kadaknath and cross breed:

- Since both Kadaknath and cross breed chickens are infected with parasite they should be treated with anti-helminthic and anti-protozoal drugs in regular basis.
- Still some parasites that are infecting cross breed chicken are not infected pure breed Kadaknath, their farming areas should be completely separated.

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