# Prevalence, identification and molecular characterization of gastrointestinal nematode parasites of goat

**THESIS**

**SUBMITTED TO**

**BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY**
**(A CENTRAL UNIVERSITY)**
**LUCKNOW**

**FOR THE AWARD OF THE DEGREE OF**

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**IN**

**APPLIED ANIMAL SCIENCES**


**SUBMITTED BY**

**SAVITA**

**UNDER THE SUPERVISION OF**

**PROF. KAMAL JAISWAL**


BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
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प्रज्ञा शील करूणा
ESTABLISHED 1996


**DEPARTMENT OF APPLIED ANIMAL SCIENCES**
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**ENROLLMENT NO.- 275/12** **Year 2021**

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# DECLARATION

I hereby declare that the thesis entitled **"Prevalence, identification and molecular characterization of gastrointestinal nematode parasites of goat"** submitted to the Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow by me for the award of degree of the Doctor of Philosophy in Applied Animal Science is an outcome of my original work and the outcome of my own efforts under the supervision of Prof. Kamal Jaiswal, Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow. It has not been submitted in part or full to this or any other University for the award of any other diploma and degree. This is also declared that the thesis is essentially free from all kinds of plagiarism.

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# CERTIFICATE

This is to certify that the thesis titled **"Prevalence, identification and molecular characterization of gastrointestinal nematode parasites of goat"** Submitted by **Savita** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University Lucknow, satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations-1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the university.

**Date:** 12/03/2021

**Supervisor**

12/03/2021

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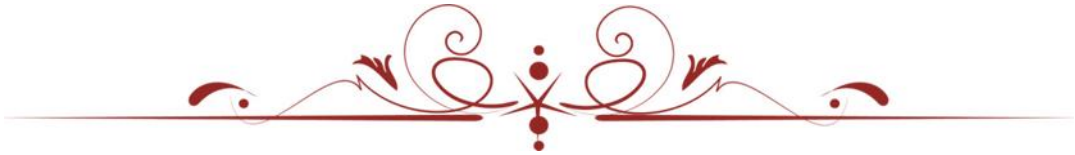
# LIST OF ABBREVIATIONS

| Abbreviations | Full Name |
|---|---|
| GIT | Gastrointestinal tract |
| ITS | Internal Transcribed Spencer |
| FAO | Food and Agriculture Organization |
| GDP | Gross Domestic Product |
| SPSS | Statistical Package for the Social Sciences |
| sp. | Species |
| spp. | Multiple species |
| M | Molar |
| Kg | Kilogram |
| G | Gram |
| Mg | Milligram |
| Mg | Microgram |
| M | Micron |
| Cm | Centimetre |
| Ml | Millilitre |
| m | Meter |
| Mm | Millimetre |
| Hrs | Hours |
| Min | Minute |
| Ph | Potential Hydrogen |
| SEM | Scanning Electron Microscope |
| Fig. | Figure |
| etc. | Et cetera |
| viz. | Namely |
| i.e. | That is |
| e.g. | For example |
| b.wt. | Body weight |
| *et al*. | And others |

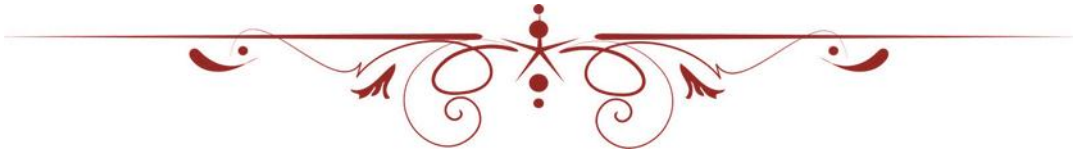
| | |
|---|---|
| Mm | Micrometer |
| Nm | Nanometre |
| µM | Micro molar |
| ng/µl | Nanogram/microlitre |
| ddH2O | Double Distilled Water |
| PCR | Polymerase Chain Reaction |
| DNA | De-oxyribose Nucleic Acid |
| RNA | Ribo- Nucleic Acid |
| PBS | Phosphate Buffer Saline |
| Sp. | Species |
| EDTA | Ethylenediaminetetraacietic acid |
| Tris –HCL | Tri-Hydro Chloride |
| TAE | Tris-acetate-EDTA |
| Etbr | Ethidium Bromide |
| CTAB | Cetyl Trimethyl Ammonium Bromide |
| UV | Ultra Violet |
| O.D | Optical Document |
| Bp | Basepairs |
| Kb | Kilobase |
| NCBI | National Center for Biotechnology Information |
| UPGMA | Unweighted Pair Group Method with Arithmetic mean |
| BLAST | Basic Local Alignment Search Tool |
| Acc. no. | Accession Number |

# SYMBOLS

| Symbols | Meaning |
|---|---|
| **%** | Percentage |
| **°C** | Degree Celsius |
| ± | More or Less analogous (Range of Standard error) |
| $\chi^2$ | Chi- square |
| ≤ | Less than or equal to |
| ≥ | Greater than or equal to |
| > | Greater than |
| < | Less than |
| **@** | At the rate |



# Chapter-1
# General Introduction



# CHAPTER-1
# INTRODUCTION

## 1.1 General Introduction

Livestock sector is a key constituent of agriculture and plays significant role in the development of world's economy and also important for rural livelihood (Iqubal, 2013). In the world, India is one of the some countries, which contributed towords the improvement of animal production and international livestock gene pool (Oldham *et al*., 2014). About 20.5 million populations are depended on livestock for livelihood and also contributed 16% and 14% to the income of small farms as against all rural households (Economic Survey Annual Report, 2018 to 2019). Two-third part of rural community depends on the livestock and also provides employment (8.8% population) in India. Livestock sector contributes 25.6% of total Agriculture GDP and 4.11% GDP (Economic survey, Annual Report 2018 to 2019). These are major limit to livestock production throughout the tropics and sub tropical regions. India possesses also one of the largest livestock wealth in the world and having quarter of the agricultural Gross Domestic Product (DAHDF, 2002).

Cattle, goat and sheep are among the first animals to be domesticated by human beings in the world (Khan and Iqubal 2011 and Tabrez 2014). Total 30 % of agricultural Gross Domestic Product (GDP) contributing to development in all countries (FAO, 2010 and Islam *et al*., 2016). Livestock sector has also contributed 512 million populations of animals in India. In India Livestock population in Gujarat, Uttar Pradesh, Assam, Punjab, Bihar, Sikkim, Meghalaya, and Chhattisgarh (15.36%, 14.01%, 10.77%, 9.57%, 8.56%, 7.96%, 7.41% and 4.34%) have increased substantially. India has World's largest livestock zone of about 535.78 million which is depended on buffaloes, cattle, sheep, goat, camel, equine, pigs, chickens, and ducks which conprises 57.83%, 15.06%, 7.14%, 17.93%, 2.18%, 1.3%, 1.2%, 4.72% and 1.94% respectvely in the world (19th Livestock census, 2012 to 2014) (Dinani *et. al*., 2018).

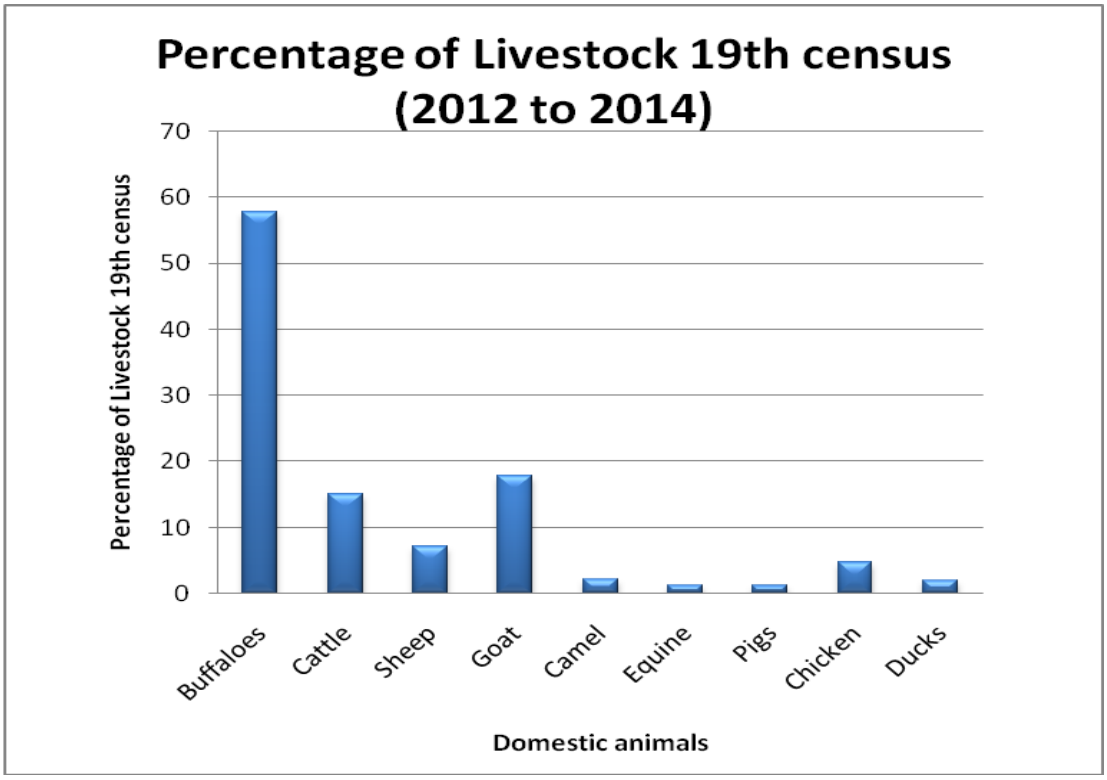



**Figure1.1 Distribution of Livestock 19th census (2012 to 2014) (Dinani *et al.*, 2018).**

Goat farming has an important role in the sustainability of rural, marginal communities and goat industries in the world, as being economically and socially highly significant at international and national levels (Morgan *et al*., 2013; Bosco *et al*., 2014 and Blackie *et al*., 2015).

Goat, a member of the Bovidae family and subfamily, Caprinae is one of the oldest domesticated species in the world. It is an important source of livelihood for small and marginal farmers as it plays an important role in providing food, fiber, manure etc. (Okpebholo, 2007 and Nizam *et al*., 2013). It is also most significant in earning substantial amount of foreign exchange by exporting meat, leather products and other products made from bones, horns and teeth per year (Alam, 1993 and Kuchai *et al.,* 2012). In livestock sub-division, the goat population plays a prominent role as it contributes to the largest population and milk production in India (Economic survey, 2011). It has an essential role in small scale farming and rural economy of developing countries (Nakanishi *et al*., 2011). The goat rearing farms are one of the important works of rural as well as national economy of India. (Nijam *et al*., 2013).

Small ruminants are the advantageous source of animal protein and primarily raised for milk, hair, leather and most important role providing source of food security (Sutar *et al*., 2010; Khan and Rehman 2009; Brahma, 2015 and Nizam, 2013). Livestock holder's livelihood depends on income generated from animals especially in the existing socio-economic conditions.

India has first rank of goat farming and second biggest goat population and also meat production in the world (Economic survey Annual Report 2018-19). It plays a significant role in providing employment and household income (Hassan *et al.*, 2011). The total number of goats and sheep were 861.9 million and 1.25 respectvely all over the world (FAO, 2008). Total meat (Chevon) production was 942.93 thousand tons procused from 86182.03 numbers of the goats. Our country has exported 21,950.71 MT of goat and sheep meat to the world for the value of Rs. 837.76 crores during the year 2015-16.

Uttar Pradesh stands 2$^{nd}$ in the country with 11.53% contributed towards meat production. The Goat contributes to about 15% of total meat production of Uttar Pradesh (Singh *et al*., 2013). Subclinical and chronic conditions resulting from reduced feed intake and decreased feed utilization efficiency are believed to be the major economic losses caused by nematode infections (Goldberg, 1952).

**1.2 Goat breeds**

A total of 351 breeds of goat are identified all over the world and in India, rarely six breeds have been identified and of which 12 breeds are indigenous and are registered which are found to have high genetic merit (meat / milk yield per animal) (Jan *et al*., 2015).

Majorly three breeds of goat (Jamunapari, Beetal and Barbari) are popular in Lucknow (Uttar Pradesh) which is also confirmed by the department of Animal Husbandry Department of UttarPradesh (Ahlawat and Kumar, 2009 and Mandal *et al*., (2014).

**Table no.1.1- Percentage of prominent indigenous breeds**

| Population as per 2012 Census | Number of prominent indigenous breeds | Number of non-Descript breeds | Percentage of non descript breeds |
|---|---|---|---|
| 135 million | 45.5 million | 82.81 million | 61.26% |


**Figure 1.2 Local breeds of goats in Lucknow (Uttar Pradesh)**

## 1.3 Parasitic infection in goat

Goats face many challenges in the form of callous climatic conditions, meagre management, uncleanliness, scarcity of fodder and infectious and non infectious diseases (Nabi, 2013). Goats are prone to various diseases (protozoan, bacterial and helminthiasis) affect the goat industry in which parasitic infection has high impact leading to the increased mortality rate of host (Dhar *et al.,* 1982 and Leiper, 1992). The bacterial and viral diseases are easily diagnosed by their experimental and clinical signs but helminth parasitic infection can not be easily diagnosed. Many studies indicate that helminth parasites are major problems of small ruminants in the world.

Parasites pose major problem across the world which is reducing the productivity of small ruminants (Gall, 1981). Chatterjee *et al.,* (2009) reported that parasites are living organisms which receives nourishment and shelter from another organism where it lives. Host is an organism which harbours the parasite. Parasites are divided into two groups ectoparasite and endoparasite. Ectoparasites lives outside the surface body of the host like tick, mice etc. Endoparasites lives inside the body of the host in the blood, tissues, body cavities, digestive tract and other organs like nematode, cestode etc.

However, the livestock are infested with many diseases, especially internal and external parasites that affect dramatically their productivity, with detrimental impact on farmer's life. Internal and external parasites seriously limit both per capita productivity and the density at which goats can be raised on farms (Blackburn *et al*. 1991, Rahman & Collins, 1990 and Abebe *et al.,* 2000).

Helminthiasis is biggest problem in the world and also leads to retardation in small ruminants (Faizal, 1999). Helminths or worms cause a wide range of health problems to both humanbeings and animals (Colley and Verde, 2001). Helminths are classified into nematode, trematode and cestode. Helminth parasitic infections remain a major problem to small ruminant production in tropics and subtropical regions (FAO, 1992). They majorly belong to endoparasites found in gastrointestinal tract of goat. Helminthic parasite infestation is the greatest significant preventive aspect of production on the goat industry (Gadahi *et al.,* 2009) due to the morbidity, cost of treatment and mortality (Nwosu *et al.,* 2007). These pathogenic infections reduce immunity among goats and which is the major reason for heavy economic losses (Garedaghi *et al.,* 2011). The incidence of parasitic diseases, anaemia, enteritis, bottle jaw is found to be prevalent may occur in different study areas (Sharma *et. al.,* 2009). Ninety percentage goats are infected with helminth parasites (Zaman *et al.,* 2012 and Al-shaibani *et al.,* 2008). These mortalities are the reason for the heavy production losses, mortalities and morbidity (Shimelis, 2011). Parasites are one of the biggest causes of production a loss where helminth parasites measured to be the mainly prevalent and significant that affects the small ruminants in world. These are responsible for both direct and indirect losses of production (Hoste, 2008 and Ahmed, 2017).

Gastrointestinal (GI) parasites most importantly include coccidia (protozoa), nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes) were found to be in this area. Surveys indicate that those nematodes are the most prevalent parasites affecting up to 95% of the goats (Rey, 1991).

Gastrointestinal nematode parasitic infection is a major menace to the goat productivity and endangers for animal welfare worldwide (Tariq *et al*., 2010). Among the gastro-intestinal nematode parasitic diseases, such as *Haemonchus*, *Trichostrnngylus*, *Cooperia*, *Oesophagostomum*, *Trichuris* and *Strongyloides* spp. are most common infectious diseases associated with anaemia, gastroenteritis resulting loss of body weight, stunted growth, bootle jaw and diarrhoea etc. that greatly weigh down the normal growth and production of goats (Soulsby, 1982). The major losses related to the nematode parasitic infections are clinical, sub-clinical, and economic assessment show that financial costs of internal parasitism are vast (Preston; Allonby, 1979 and McLeod, 1995). They cause lower productivity, mortality and economic losses, thus affecting the income of small holder dairy farming communities (Sykes *et al*., 1994; Khan *et al.*, 2010 and Namavari *et al.*, 2011).

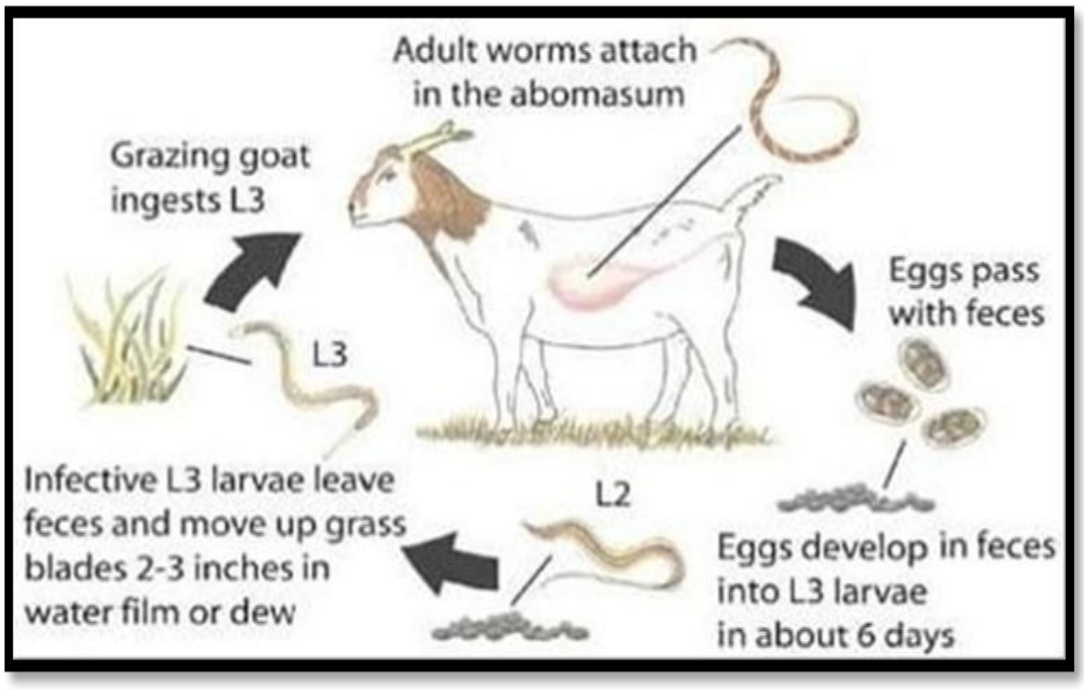



**Figure 1.3 Life-cycle of gastrointestinal nematodes parasites in goat (McCollough *et al*., 2019)**

The life cycle of the gastrointestinal nematode parasites are completed with direct and do not require any intermediate hosts, which be appropriate to all of the economically significant strongylid parasites of small ruminants (Hansen and Perry, 1994 and Urquhart *et al*., 1996). Whole life cycle is completed within 15 days in favourable conditions at appropriate temperature and humidity. Growth and development occurs within the faecal accumulation and eggs are embryonated and hatched out within 2 to 3 days, these stage of larva are called first stage larvae (L1), which in turn moult into 2$^{nd}$ stage larvae (L2), flaking their protective cuticle are produced. After 4 to 6 days L2 moult into third-stage larvae (L3), but maintain the cuticle from the earlier moult. The L3 represent the infective stage, and these move about on to surrounding vegetation where they become available for ingestion by grazing animals. Following ingestion, the L3 larvae pass to the gastrointestinal tract of goat.

The trichostrongyle worms (L3 stage) penetrate the epithelial layer of the mucus layer (*Haemonchus* sp. and *Trichostrongylus* sp.). L3 moult within 2–3 days to become 4th-stage larvae (L4), which stay put in the mucous layer. Finally, the L4 emerge and moult to become young adult parasites.

**1.4 Environmental factors**

In severe cases, nematodiasis causes losses of growth and production due to mortalities, however the effect on production depends on various aspects the parasite species, intensity and abundance of parasitic burden. Other factors that cause diseases and eventually pose highly economic loss are climatic factors, physiological status of the host and husbandry preparation (Al-shaibaniI *et al*., 2008).

According to Pal and Qayyum, (1993) the parasitic infections depend on different climatic conditions like quality and quantity of pasture, humidity, temperature and grazing behaviour of the animals. However specific parasites may be distributed all over the world and they have varied impact according to production system, geoclimatic conditions and management (Kennedy and Harnett, 2011) which are mostly associated with research institutions and government (Amulya *et al*., 2015).

The seasonal fluctuations increase and decrease due to the infective stages of larva which is prejudiced by the contamination of the pasture. The biotic factors are responsible are density of stocking, and the immune status in the goat (Hansen and Perry, 1994 and Urquhart *et al*., 1996).

The development, transmission and survival of the various stages of larva of nematode parasites are prejudiced by climatic factors within the faecal sample and herbage. These include climatic factors like temperature, humidity, rainfall, soil and moisture. Under humidity and temperature (Optimal condition), the development and growth of larvae of most species is 85% desiccation (Donald, 1968; Tembely, 1998 and O'Connor *et al*., 2006).

Seasonal variations of summer, monsoon and winter and effects of various factors are responsible for the worm burdens in the hosts. This seasonal variation of parasite population dynamics has been described in a number of studies in many countries (Assoku, 1981; Van Wyk, 1985; Fakae, 1990; Pandey *et al*., 1994; Tilahun, 1995; Nginyi *et al*., 2001 and Debela, 2002). In general, express translation of eggs through to L3 occurs throughout most of the summer and rainy seasons, and grazing host and acquires the highest infections during these times (O'Connor *et al*., 2006).

Climatic conditions provide suitable environment for the transmission of parasitic infections (Mohanta *et al.,* 2007; Varadharajan and Vijayalakshmi, 2015). Meteorological observations have been used to find the climatology of rainfall, temperature, humidity and wind in the study area (Singh *et al.,* 2012). Climate changes have been implicated as a dynamic force for parasites, and made relationship between host and parasitic interaction (Naomi, 2012). Generally, rainfall, warm and humid conditions are also responsible for the high parasitic infection. The increased parasitic infections in recent years has been recognized in different climate conditions (summer, monsoon and winter) (Armour *et al.,* 1980; O'Connor *et al.,* 2007; Raza *et al.,* 2009 and Kedarkarki *et al.* 2012). Clinical and subclinical parasitic infestation also increase mortality rate, weight loss, lower milk yield, abortion, infertility, increased veterinary costs, and heavy infections further leading to host mortality of goat (Sood, 1981 and Wilson, 2007; Arshad, 2012 and Raza 2014).

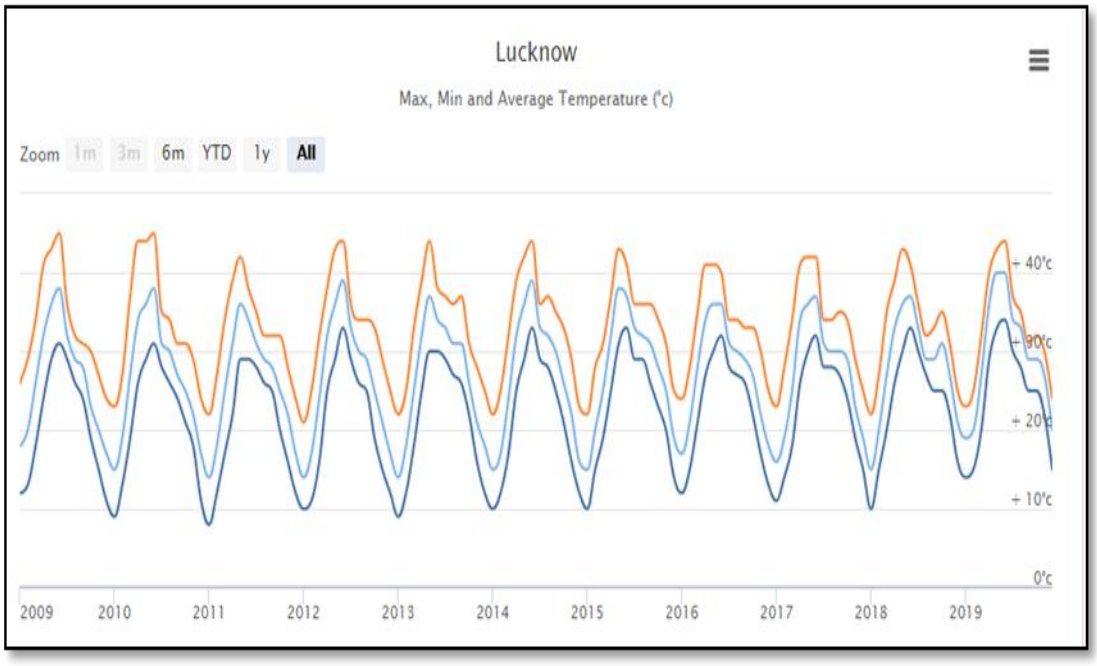



**Figure 1.4 Metrological identification of temperature during 2010 to 2019 at Lucknow (Uttar Pradesh)**

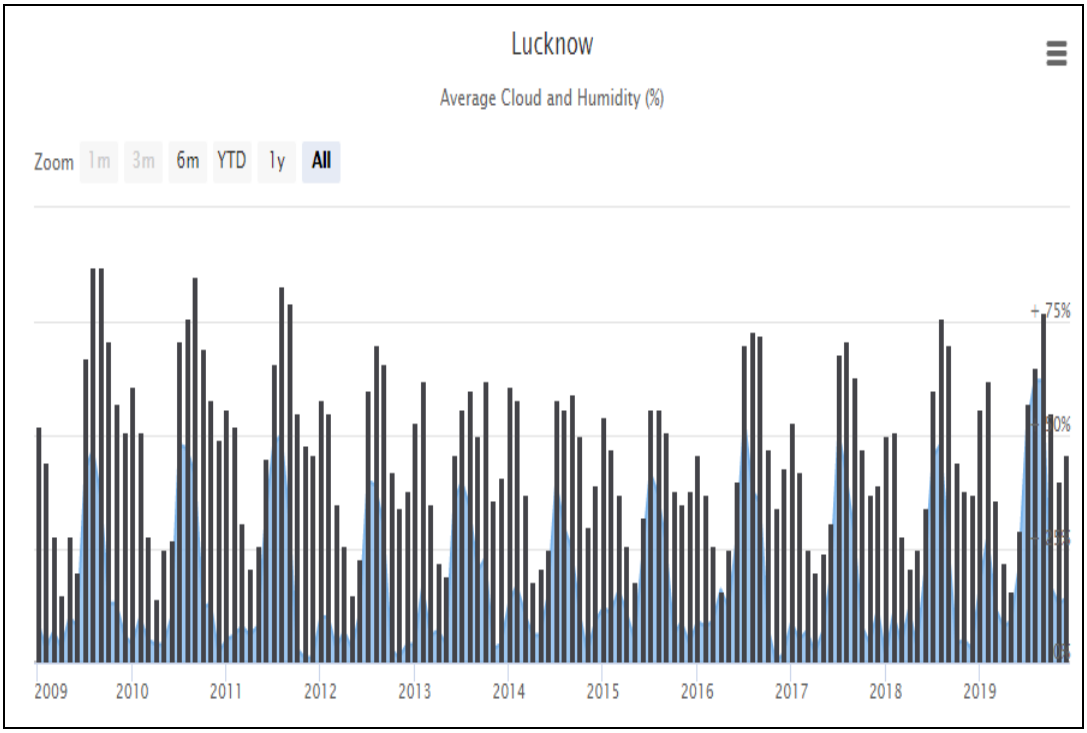



**Figure 1.5 Metrological identification humidity during 2010 to 2019 at Lucknow (Uttar Pradesh)**

Surveys indicate that mortality rates of host are infected with gastrointestinal nematode parasites (Rey, 1991) are exceed 40% while weight losses in the range of

6 to12 kg/year/animal may occur (IEMVT, 1980) because parasites are blood sucking. Decreased immunity leads to productivity losses reducing the feed intake and decreased feed utilization which are related with subclinical and chronic conditions, leading to heavy economic losses (Holmes, 1993 and Gatongi, 1996).

**1.5 Species of parasites**

In India approximately 300 species of helminth parasites are found and new species of parasites are being commonly discovered (Singh *et al*., 1977). Nematode parasitic infection of gastrointestinal tract is one of the main problems in goats which are categorized clinically and subclincally by anemia, enteritis, hypoproteinaemia, dehydration, anorexia, diarrhoea, abdominal distention and emaciation, lower blood glucose and death. These diseases are responsible to affect the body weight, growth, yield and reproductive recital of host leading to economic losses to farmer and the nation in general and also comprise a major obstacle to competent and gainful livestock production (Sharma *et al.,* 2014, Maiti *et al*., 1999, Junaidu and Adamu, 1997, Owhoeli and Elele, 2014). The losses of goat production due to diseases at nation wide rank were approximate at Rs. 11,720 million per annum.

*Haemonchus contortus* and *Ostertagia ostertagi* are blood sucking parasites, cause major economic losses (0.05ml of blood/worm/day) campared to other parasites in the worldwide (Soulsby, 1986). *Trichostrongyle* infection (Trichostrongylosis) is a very infectious parasite in the veterinary which is causes anaemia, weight loss, poor wool, and milk production, bottle jaw and weakness respectively. *Oesophagostomum* spp. produces nodule formation in intestinal tract. *Trichuris ovis* and *Bunostomum* spp. are allied anaemia, necrosis, haemorrhage, diarrhoea and caecal mucosa has been reported in severe infections. In heavy infestation of parasites in the goats, they may die due to development of severe interstitial emphysema, respiratory failure and pulmonary oedema. Goats are infected with mixed parasitic infections of several nematode species (Agyei *et al*., 1998). The most prevalent genera of gastrointestinal nematodes parasites are reported in table number-1.2 (Assoku, 1981).

**Table no. 1.2- Gastrointestinal nematodes parasites and their eggs (Nabi, 2013).**

| Species of Parasite | Predilection Site | Size of Male | Size of Female | Time required for L3 Development | Morphological Characters | Egg Measurement |
|---|---|---|---|---|---|---|
| *Haemonchus* | Abomasums | 10-20 Mm | 18-30 mm | 4-6 days | Female have Barber pole appearance. Male bursa dorsal dorsal lobe with Y shaped ray. | 70-85 by 41-48 µm |
| *Trichuries* | Large intestine | 50-80 Mm | 35-70 Mm | 2 to 8 days | Eggs are brown barrel shape | 70-80 by 30-42 µm |
| *Oesophagostomum* | Large intestine | 3-4.5 Mm | 4.5.5 Mm | 2 to 5 days | Eggs oval in Shape | 60-70 by 30-40 µm |
| *Trichostronylus* | Abomasums | 4-5.5 Mm | 5-7 Mm | 4-6 days | Eggs Oval in shap and pointed both end | 79-101 by 39-47 µm |

## 1.6 Identification of nematode eggs

Feacal examination techniques have revealed occurrence of gastrointestinal eggs (Kennedy, 1982). This examination means the detection of the parasites and their eggs from faeces, but generally it refers to the examination of helminths eggs and oocysts. However, mostly parasites produce similar eggs and oocysts which cannot be identified easily at the species level in many nematode eggs from host (Minami et al., 2001 and Demelash, 2016). Identification of helminth parasites and eggs are important constraints in determining the cause of the disease and load of parasitic burden. The examination of faecal matter is providing evidence of qualitative and quantitative method for identification of the parasitic eggs in the gastrointestinal tract in the host. (Kirberger *et al*., 2007).The advantage of examination of fecal sample is that simple and less costly, and its disadvantage is that certain parasites produce similar eggs (Foreyt, 2001).

Sedimentation and flotation techniques are used to separate larvae and egg from faecal samples and is used mostly to examine and identification of eggs of parasites of nematode, cestode and trematode parasites. Therefore, these methods are allowed by microscopic detection and quantification (i.e., number of eggs/gram) of

eggs. If faeces are not fresh the flotation procedure should be used (Bowman *et al*., 2003).

### 1.7 Signs of parasitism

Transmission can be by both passive (food and water) and active by the cutaneous incursion of parasites worm (Dimitrijevic *et al*., 2012). Some Infectious parasites may also migrate to the udder due to systemic circulation before birth, so galactogenic transmission is possible in the host (Sibalic and Cvetkovic, 1996).


**Figure 1.6 Signs of parasitism in goat (A) Neck infection (B) Bottle jaw (C) Anemia (D) Rough hair coat (E) Diarrhea (F) Weakness and Death.**

## 1.8 Control of nematode parasites

The control of gastrointernal parasitic infection in goats by some methods like enthelmintics, animal management, ethnovetenary plant extract, drugs and improved pasture management can be used to kill the parasites and break the life cycle of parasitic infection.

### 1.8.1 Pasture Management

Pasture management is a key component of gastrointernal parasitic infection. The grazing behaviour of goats makes them much more vulnerable to parasitic infection than other species. Pasture management can be conducted to reduce the number of parasitic infection. The best way to avoid parasitic problems is to keep away from overgrazing and to put into practice a rotational grazing system. These processes can effectively reduce the chance to ingest larvae and easily reduce infection of parasites.

### 1.8.2 Use of drugs and plant extracts for control of parasitic infestation in goats

The control of gastrointestinal helminths is mostly based on the regular use of anthelmintics. In the humid and rainy seasons, the parasitic burden is increased and therefore anthelmintic treatments are most important to reduce the infection and achieve the satisfactory weight of goat and increase the economic value in industries (Allonby and Urquhart 1975; VanWyk *et al*., 1999 and Waller, 1997). Reduction of infestation of gastrointestinal helminths parasite, through wide-ranging active compounds against the nematodes, trematodes and cestodes and, their larval stages is effective (Probert, 1994).

Some drugs available are used to kill the parasitic infection in goats but some drugs are resistant, like albendazole and benzimidazoles, avermectins and imidothiazoles are helpful to remove infection. These drugs are used as anthelmintic to control and treat gastrointestinal parasitic infection in goats. Benzimidazoles are also drugs which contain the anthelmintic fenbendazole, albendazole, oxfendazole, mebendazole, and oxibendazole. Benzimidazoles, avermectin, ivermectin and moxidectin are main drugs and very effective against helminth paracitic infection. (Coleby, 1993).

Anthelmintic plants extract are potential and promising alternative used as commercial against parasitic infection. Studies from various parts of the world have shown that some plant species *(Mangiferaindica* (Mango)**,** *Azadirachtaindica* (Neem), *Eugenia caryophyllata* (clove*), Ananas comosus* (pin***e***apple) etc. is quite effective in reducing the infection of parasite in small ruminants (Anderson *et al*., 1965).

### 1.8.3 Diagnosis of gastrointestinal nematode infections

Gastrointestinal nematode infections can be effectively controlled are accurate by diagnostic techniques with the help of control and treatment which may be direct and indirect (Gibson *et al*., 1976). A variety of methods and modifications have been described for such diagnosis and standardization of techniques, such as egg or larval counts, pasture larval counts and larval cultures (Githori *et al*., 2002). Variety of techniques, such as direct smear, Baermann technique, sedimentation, flotation, McMaster, molecular technique, postmortem examination, and pasture larvae count (Biu *et al*., 2014) in laboratory and field diagnosis. Correct diagnostic techniques are important to identify parasite species level (Bayon, 2005).

Therefore, the present investigation is planned to carry out a study on "Prevalence, Identification and molecular characterization of gastrointestinal nematode parasites of goat".

# *Objectives*

## Objectives

The proposed study examines the prevalence, identification and molecular characterization of gastrointestinal nemaotode parasites in goat (*Capra hircus*) at Lucknow. The following objective were proposed for the study-

1. To study the prevalence of gastrointestinal nematode parasites in goats at Lucknow.
2. To study the morphological identification of gastrointestinal nematode parasites of goats.
3. To determine the molecular characterization of gastrointestinal nematode parasites of goats.

# CHAPTER-2

# REVIEW OF LITERATURE

# CHAPTER-2
# REVIEW OF LITERATURE

Goat is important livestock species all over the globe and especially in tropical and subtropical regions (Akhtar *et al*., 2002). It has a pivotal role in small scale farming and rural economy of developing societies by generating employment and supplementing house hold income. Goats are primarily raised for milk, meat, hair and leather production. Parasitic nematodes of gastrointestinal tract (GIT) are the main constraints to goat production all over the world and are a significant health issue in areas with poor sanitation and management. In addition, different countries have reported varied parasite species from their microenvironment where livestock are formed (N'Depo *et al*., 1987; Bishop and Stear *et al*., 2001).

Goats harbor a variety of GIT parasites that affect the production as well as growth of the animals. A voracious blood sucking parasite found in abomasum causes anaemia, diarrhoea, loss of weight, oedema, recumbency, severe debility and ultimately death. Furthermore, stress, poor nutrition and coincidental diseases may aggravate the condition of affected animals. Heavy economic losses due to reduced productivity, mortality and parasite control measures are recorded in nematode parasites. Caprine parasite gastrointestinal infection is worldwide in distribution (Barker *et al*., 1999 ; Chiejina *et al*., 2002 and Miller *et al*., 2005). The bacterial and viral diseases are easily diagnosed by their clinical signs but helminth parasitic infection have no clinical signs when less in number and thus act as one of the major cause losses of growth and production. Faizal *et al*., 1999, studied that 1/3rd growth retardation in ruminants due to helminth parasitic infections. The productivity of goats and sheep is constrained by parasitic infections due to incresed rate of mortality (5-10 % ) and morbidity (10-20 % ) (Dhar *et al*., 1982 and Herlich *et al*.,1978). Total 95% sheep and goats are infected in the tropics areas with (Rey *et al*., 1991). Mortality rates in herds may exceed 40% while weight losses and causes of economic losses (Holmes *et al*., 1993; Gatongi *et al*, 1996). The parasitic infected animals increase their rate of metabolic and reduce the metabolic energy used for production, as the parasites use their nutrients, damage some vital organs and cause animals to become more susceptible to other pathogenic agents. The higher incidence of

helminth infections in goat in a grazing system lowers productivity, leading to economic losses. (Skykes *et al*., 1992).

Therefore, this present review of litrature hightlight the gastrointestinal parasites on basic of prevalence, identification and molecular characterization of parasites and impact in their host (*Capra hircus*).

**Mattos *et al.*, (1991)** studied nematodes parasites found in gastrointestinal tracts in cattle, sheep and goat in Oriximina, Brazil. Eight species of parasites were reported namely, *Haemonchus contortus, Haemonchus similis, Trichostron gylusaxei, Trichostrongylus columbriformis, Cooperia curticei, Cooperia punctata, Oesophagostomum venulosum and Bonostomum trigonocephalum.*

**Mark (1996)** idenrtified the small-subunit rRNA gene sequences of Neospora spp. and other apicomplexa coccidian with the help of oligonucleotide primers COC-1 and COC-2. These primers were used for PCR amplification of conserved sequences. Southern blots were also used for amplification produces DNA from the other coccidian parasites tested. The PCR amplification detect the 300bp spp of *Neospora* spp. These results revealed that PCR and probe hybridization system, serology and immunohisto chemistry for the diagnosis of Neospora infestation in bovine or primate fetuses.

**Valcarceli and Romero (1999)** examined 322 gastro-intestinal tracts of conventionally reared goats from a dry region of Central Spain. Gastrointestinal nematodes parasites were observed with *Teladorsagia trifurcate* and *Teladorsagia circumcinta* being the most prevalent parasites, followed by *Nematodirus filicolis* and *Trichostrongylus vitrinus*.

**Jithendran and Bhat (2001)** reporeted gastrointestinal parasitic infection in goats and sheep at Himachal Pradesh, India. Observerd parasitic prevalence infections were found in sheep and goats respectively as follows: *Fasciola (*9.6 and 8.8%), *Amphistomes (*3.8 and 2.5%) *Dicrocoelium (*7.2 and 2.5%) *Schistosoma (*1.2% and 0.6%) *Moniezia (*2.7% and 1.3%) *Strongyles (*91.6 and 100%) *Strongyloides (*4.8 and 5.1%) *Dictyocaulus (*1.2 and 1.3%) and *Trichuris (*14.3 and 1.3%).

**Sharkhuu (2001)** studied 236 goats from Mongolia for parasitic infection in goats. Only 39 helminth parasites species were found belonging to three classes, fourteen families and twenty three genera. Helminths parasitic prevalence in goat was reported for, 4 seasons 3 geographic area and 3 age groups. During the study observed that March month (average 1335.3±405.3) was more infected than November month (54±18.6) and three parasitic species were found during study period *Marshallagia, Nematodirus* and *Ostertagia*.

**Mazyzd and El-Nemr (2002)** studied the gastrointestinal parasites in sheep goats and cattle in North Sinai Governorate, Egypt. observed pathology and finding of internal parasites in ruminants. The purpose of their study was to overview the gross pathology and diagonosis of gastrointestinal and other parasites in ruminants, with particular emphasis on the economically important parasites of sheep, goat and cattle. In this study period observed that three species were more prominent in this area, *Fasciola spp*. (12.7%), *Moneiza expansa (*12.8%) and *Trichuris ovis (*4.59%).

**Regasa *et al*., (2004)** conducted studies on epidemiology of endoparasites of ruminants in Western Oromia, Ethiopia. This study was carried out parasitic prevalence of intestinal parasites as 84.1% of goats. Nematodes parasites are most important endoparasites in this study area of group *Strongyle, Eimeria which* were found to be most prevalent parasites encountered in this area.

**Dhand *et al*., (2004)** reported an incidence of fasciolasis disease in goat and sheep in Punjab. In this study was found that 70 goats and 50 sheeps of different age groups were found that suffering from diarrhoea with high fever. Among these animals 5 goats and 40 sheep died before the investigation. *Fasciola gigantic* was recovered on post mortem examination.

**Mbae *et al*. (2004)** studied 1106 sheep and goats in Kenya for nematode parasitic infections. Young animals were found more infected than older. The faecal egg examination was significantly higher ($p<0.05$) in monsoon seasons in both animals (sheep and goats). *Haemonchus contortus* was the most prevalent nematode parasite in this area as comapared to *Trichuris* spp. and *Nematodirus* spp. Parasites.

**Sheikh *et al*., (2004)** studied fasciolasis in sheep in Kashmir valley. They examined 1150 faecal samples from the infected rectum of sheep of hilly migratory

groups. Flukes were identified in the local breed of sheep and comparison study was done for prevalence in altogether 389 livers. They found both mature and immature flukes.

**Pawel Gorski *et al.*, (2004)** studied the cross-sectional prevalence of endoparasites (protozoan, tapeworms, nematodes, flukes and cestode) in small ruminants from different zones of Poland. Total 400 sheep and 180 goats were examined that the infections was higher in goats as compared to the sheep. *Muellerius capillaris* (lungworm) parasites were most prevalent in goat less in sheep. Female goats were also more infected with lungworms (*Muellerius capillaries)* and less in males. The most prevalent nematode parasites were *Trichostrongylus* spp in both hosts.

**Sangvaranond *et al.*, (2005)** studied 358 samples of goats (75 male goats and 283 female goats) examined for eggs and cysts of internal parasites by using sedimentation method. Most of the examined goats were crossbred goats (Thai native and Anglo-nubian breeds). The prevalence and intensity of parasitic infection and cysts of intestinal parasitic protozoa were *Paramphistomum* (rumen flukes) (9.22%), *Eurytremapan creaticum* (0.84%), *Moniezia expansa* (2.23%), *Moniezia benedeni* (8.38%), *Strongylids* (93.85%), *Strongyloides* spp. (7.26%), *Trichuris* spp. (6.42%), *Entamoeba cysts* (71.23%), *Giardia cysts* (2.51%) and unsporulated *Coccidian oocysts* (43.30%). Common helminthic infestation of the goats in Saraburi province was disease of goat strongyles. Most parasitic infection was two or more species of helminths in goats. *Haemonchus* spp. and *Oesophagostomum* spp. were the dominant nematode genera showing infecting the host. This study indicated that 81 % of farmers take care of their livestock by feeding them with supplements. The fecal egg counts from cattle showed that helminth infections in this region are still under control even though helminthosis seems to be a problem in small-stock, since EPGcounts of more than 1000 was found.

**Chaudary *et al.*, (2007)** investigated the prevalence based on seasonal variation, in northern Punjab, Pakistan during December 2004 to January 2006 in sheep and goats. Faecal samples were collected from different breeds of 968 sheep and 961 goats. Results investigated and it was reported that the infection was significantly higher ($p<0.05$) compared to sheep and goats. The infection level was

recorded during rainy season (July to October) and low infection in December to May.

**Sissay *et al*., (2007)** studied the gastrointestinal parasites in small ruminants (655 sheep and 632 goats) in eastern Ethiopia. 8 sheep and goat owned by farmers and received breeding stock from the HU for the FECRT. 50 local breed goat and sheep, 6 to 9 months old, were divided into five groups of treatment: ABZ, TET, ABZ + TET, IVM.

**Menkir *et al*., (2007)** carried out a two year epidemiology study of helminthes parasitic infection of small ruminants. The study involved the collection of 655 sheep and 632 goats from 4 in eastern Ethiopia. A further more detailed epidemiology study of gastrointestinal nematode infections used the Haramaya University (HU) flock of 60 Black Head Ogaden sheep. The parasitological data included number of nematode eggs per gram of faeces worm culture, packed red cell volume (PCV), and FAMACHA eye-colour score estimation, along with many others performance like body weight of animals. Toat thirteen species of nematode parasites and four species of flukes present in the sheep and goat. *Haemonchus contortus* parasites were most prevalent (65–80%), followed by *Trichostrongylus* spp.

**Jani (2008)** reported that overall 62.5 faecal matters were parasitic infected out of 40 Indian elephants (*Elephas maximus*). *Fasciolia* spp. (15.00%) was most infected as compared to other parasitic infection. About 48 percent elephants loose faeces grossly and manifested drying out along with a habit of earth defeat.

**Pathak and Pal (2008)** reported an overall 85.22% parasitic infection in gastrointestinal parasites of goats. Seasonal studies was showed that highest parasitic infection in monsoon with 94.60%, summer with 87.50% as compared to winter with 63.15%.The prevalence of different parasites were *Paramphistomum* spp. (80.68%), *Cotylophoron* spp. (45.45%), *Oesophagostomum* spp. (30.68%), *Trichuris* spp. (27.27%), *Haemonchus* spp. (26.13%), Moniezia spp. (17.04%), *Trichostrongylus* spp. (5.68%0, *Bunostomum* spp. (5.68%), *Avitellina* spp. with (3.40%) and *Cooperia* spp. (3.40%).

**Rajapakse *et al.*, (2008)** collected 218 gastrointestinal tracts and examined the parasitic infection in Sri Lanka during. Total 217 gastrointestinal tracts were found infected in during study periods. Five species of nematode parasites were examined in the abomasums and intestines of the host. They were *Oesophogostomum columbiamum* with 88%, *Haemonchus contortus* with 81%, *Trichostrongylus columbriformis* with 76%, *Trichostrongylus axei* with 59% and *Trichuris ovis* with 59%.

**Tariq *et al.*, (2008**) reported seasonal epidemiological nematode parasitic prevalence in different age, breeds and gender of sheep through faecal study during the period of 2 years in Kashmir valley, India. Overall study was completed in two parts, first 1 year infection was 64.76 % and second 58.37% of the 1533 sheep. Found different parasites were *Haemonchus contortus*, *Ostertagia circumcincta*, *Chabertia ovina Bunostomum trigonocephalum*, *Trichostrongylus spp.*, *Nematodirus spathiger*, *Oesophagostomum columbianum*, *Trichuris ovis* and *Marshallagia marshalli* (59.6%, 38.0%, 37.7%, 37.7%, 33.9%, 29.4%, 28.4%, 23.5% and 22.1%). Local Kashmiri breed reported low infection as compared to other breeds ($p > 0.05$). Kids were more infected than adult hosts. Gender wise prevalence was showing higher infection in males than females and statistically analysis showed no significant data ($p > 0.05$).

**Mijaz *et al.*, (2008)** carried out studies on goat and found out the infection rate of gastrointestinal tract (GIT) helminthes and the results revealed an overall infection rate of GIT helminth which was 63.33 % in goats, they compared the class wise infection rate, highest infection rate of nematodes (42.67%) was observed, followed by trematodes (16.67%) and cestodes (4%).

**Shirale *et al.*, (2008)** reported 350 faecal samples of cattle collected from Western vidarbha region around Akola. Out of total 232 sample, 62.29% had single and 6.00% had mixed infection were examined and confirm helminth parasitic infestation. Total 9 species of helminths, *Strongyle* spp., *Strongyloides* sp., *Trichoderma* sp., *Haemonchus* sp., *Trichuris* sp., *Trichostrongylus* sp., *Moniezia* sp. , *Facsiola* sp. and *Coccidia* sp. (19.39%, 11.14%, 8.28%, 6.57%, 5.42%, 4.85%, 4.18%, 3.71% and 3.14%) were found helminths parasites in cattle. Seasonal studies of parasites were reported to be higher infection in rainy and lower in winter season.

*Strongylus* sp. was the most parasites in all the seasons. The higher incident of parasitic infection observed of nematodes compared to the cestodes and trematodes.

**Gadahi *et al.*, (2009)** examined overall 254 (63.50%) found infected out of 400 faecal samples in Rawalpindi and Islamabad. Among 48 sheeps (53.33%) and 206 goats (66.45%) were found infection out of 90 sheeps and 310 goats respectively. Five species were found in during study periods, *Trichuris* sp. with 40.00%, *Haemonchus* sp. with 28.88%, *Coccidia* sp. with 27.77%, *Nematodirus* sp. 11.11% and *Fasciola* sp. 4.44% respectively.

**Qamar *et al.*, (2009)** reported the epidemiological studies of Burble pole worm (*Haemonchosis)* in goats and sheep at different regions of slaughter houses, veterinary hospitals and livestock farms in accordance to different environmental factors in Punjab province, Pakistan. Infection rate of parasites were 38.04, 36.83, and 35.44%, respectively in goats and sheep at veterinary hospitals and Livestock farms. Overall prevalence high in rainy season (43.69%) as compared to autumn (38.46%), spring (37.12%), the lowest (28.79%) were during winter season. Gender wise infection has showed not much difference and age wise study indicated that >6 months were prone to higher infection than <9 month of age.

**Raza *et al.*, (2009)** reported a study on rumen of goats (42), cattle (34), sheep (14), and buffalo (10) to determine the prevalence of parasitic infection of adult *Paramphistomum cervi*. They found an overall prevalence of 22%. In this survey, prevalence of *Paramphistomum cervi* was recorded to be highest in sheep and lowest in cattle.

**Tiwari *et al.*, (2009)** reported the prevalence of intestinal parasites in pigs of Grenada, a cross-sectional study was undertaken. During July 2009, carpological study was accepted out on 221 pigs from 16 farms. The overall prevalence of intestinal parasites was 68.78% (95% CI, 62.67 to 74.89%). Four parasites were identified including *Oesophagostomum spp., Strongyloides spp. Trichuris suis* and *coccidian spp*. Mixed infections were showed 6/10 (60.0%) in small herds and 5/6 (83.3%) in large herds. There was no significant ($p>0.05$) difference between infection rates on larger and smaller pig farms ($p > 0.05$). There was also no association between infection rate and age group on either smaller farms ($p = 0.12$)

or larger farms (p = 0.06). There was no evidence of infection with *Ascaris suum*. The results of this study provide baseline information about intestinal parasites of pigs and preventive methods currently in use in Grenada.

**Qamar *et al.*, (2009)** reported epidemiological studies of *Haemonchosis* disease observed in gastrointestinal tract of goat and sheep, which were collected from the different regions of slaughter houses, livestock farms and veterinary hospitals according to on different weather conditions in Punjab province (Pakistan). Infection rate of haemonchosis disease was most prevalent (35.44, 38.04 and 36.83%) in sheep and goats.

**Ikpeze and Nzemeka (2009)** reported that gastrointestinal tracts of goats was infected by differential worm count of helminths at Ibusa abattoir, Delta State, Nigeria, during the period of February and April, 2008. Total 1354 goats slaughtered were examined for infected intestines and 141 GIT (10.41%) were randomly identified, 34 (29.82%) and 19 (16.67%) having mild, moderate and severe helminthiasis, respectively. Worm count data was revealed three species of Trematodes *Dicrocoelium dendriticum* in the bile ducts and gall bladder, *Fasciola gigantica* (liver and bile ducts) and *Paramphistomum cervi* (rumen and reticulum). 3 species of Cestodes composed of *Avitellina centripunctata*, *Moniezia expansa* and *Moniezia benedini* in the ileum, and Nematodes species were examined *Gaigerapa chyscelis* (duodenum), *Haemonchus contortus* (abomasum), *Trichostrongylus axei* (abomasum), *Oesophagostomum columbianum* (colon) and *Strongyloides papillosus* (ileum) in criminated in parasitic gastroenteritis (PGE) in ruminants. Total infection was examined was 321.75, with the stomach recording of 125.75 (39.08%), intestines 130.75 (40.64%) and liver with bile duct and gall bladder 65.25 (20.28%). nevertheless, *Haemonchus contortus* contributed 18.10% to the total worm burden, followed by *F. gigantica* (14.37%), *T. axei* (13.83%) and *M. expansa* (10.96%), *M. benedini* (8.70%), *A. centripunctata* (7.85%), *P. cervi* (7.15%), *D. dendriticum* (5.91%), *S. papillosus* (5.75%), *O. columbianum* (4.43%), and *G. pachyscelis* (2.95%).

**Khan *et al.*, (2010)** observed and confirmed the prevalence of gastrointestinal (GI) parasitic infection helminths in the domestic animals at Punjab (Pakistan). In this study has been concluded total 1,140 cattles, 1,140 buffaloes, 660

goats, 840 sheep, and 156 camels and faecal samples were identified with the help of floatation technique. The prevalence of helminths parasites were significantly higher (P<0.05) in sheep (44.17%, 371/840) as compared to other livestock. Sheep were followed in order by goats (40.15%, 265/660), cattle (33.68%, 384/ 1,140) and buffaloes (39.82%, 454/1,140).

**Sultan *et al.*, (2010)** carried out a study and found 98 (51.9%) helminthes infection out on 189 intestine of sheep which they were identified as fasciola species *Paramphistomum cervi*, *Moneizia expensa, Avitellinacenteri punctata*, *Cesticurcuste nuicollis, haemonchus contortus,* and *graphidiops spp*. They reported that cestodes infection was high as compared to nematodes while infection due to the trematodes.

**Akhter *et al.*, (2010)** this study was conclude the prevalence of gastrointestinal nematode parasites in goats (n=1065) in Hyderabad using qualitative and quantitative coprological examinations. Results revealed that 43.10% (459) goats were infected with different species of nematodes parasites including *Haemonchus contortus* (14.65%), *Trichuris ovis* (8.17%), *Trichostrongylus axei* (7.61%), *Trichostrongylus colubriformis* (6.76%), *Oesphagostomum columbianum* (5.35%), *Ostertagia circumcincta* (5.35%), *Chabertia ovina* (4.79%) and *Strongyloides papillosus* (4.51%). Infections with mixed species of nematodes were recorded in 6.54% (n=30/459; *T. ovis* + *H. contortus*), 5.23% (n=24/459; C. *ovina* + *H. contortus*), 5.88% (n=27/459; S. *papillosus* + *C. ovina*), and 12.42% (n=57/459; *O. circumcincta* + *T. ovis*) goats. Of the total infected intestines (n=459); 51.4, 38.3 and 10.2% goats had light, moderate and heavy infections, respectively. The prevalence, nature and intensity of the helminthiasis in goats warrant an immediate attention to devise strategies for its control to reduce the production losses.

**Hassan *et al.*, (2011)** reported the study of prevalence of ecto and endopatasites in goat at Chittagong district and Bangladesh study was done during the February to May (2006). The overall prevalence (N=317) of gastrointestinal parasites were 63.41% in black bengal goats. *Strongyloides* spp. were most prevalent parasites as compared to *Moniezia* sp. and *Capillaria* sp. Faecal sample estimate shows, 36.95% and 13.56% were infected with infection of parasites eggs. Age-wise studies was showed that highest infection in older goats (> 24 month) by

endoparasites as compared to younger (< 24 month). In this study significantly higher data (P< 0.05).

**Godara *et al*., (2011)** studied the efficiency of fenbendazole, ivermectin and levamisole was chequered in comparison to untreated controls in 20 Jamunapari goats, naturally infected with gastrointestinal parasites. Faecal sample examination at day exposed an egg per gram (egg/gram) of 930 ± 175.1, 1350 ± 421.1,1060 ± 224.9 and 800 ± 279.7 in group A, B, C and D, respectively having 5 host each. The results of larval culture examination was revealed that the presence of parasites *Haemonchus* spp., *Oesophagostomum* spp., *Trichostrongylus* spp., *Strongyloides* spp. and *Bunostomum* spp.. Faecal egg counts of the host treated with the drusgs with concentration, fenbendazole (23.66%), levamisole (63.70% ) and ivermectin (98.11%) (Group A, group B and group C)**.**

**Edosomwan and Shoyemi (2011)** studied two hundred and seventy faecal maters samples reported for gastrointestinal helminth parasites using floatation and sedimentation techniques in order to compare the gastrointestinal helminth parasites infecting cattle and goats. The result from the faecal examination of cattle revealed the presence of twelve (12) gastrointestinal helminth parasites, namely; *Trichuris globulosa, Capillariab ovis, Moniezia benedeni, Dicrocoelium hospes, Toxocaravi tulorum, Ostertagia ostertagi, Trichostrongylus columbriformis, Fasciola gigantica, Strongyloides papillosus and Taenia saginata*. The result for goats revealed the presence of five (5) gastrointestinal helminth parasites, which were as follows: *Trichuris ovis, Capillaria* sp., *Strongyloides papillosus,* and *Moniezia expensa*. Of the 135 faecal samples examined of cattle, 76 (56.30%) were positive using the floatation technique while 52 (38.52%) were positive using sedimentation technique. For goats, using sedimentation technique 9 (6.67%) of the 135 samples were positive while 18 (13.33%) were positive, for floatation technique. A statistical significance was found for cattle using floatation and sedimentation techniques ($p<0.001$), however, there was no significant difference between sedimentation and floatation techniques for goats. From the study, floatation technique was more effective for detecting helminth parasites eggs in faecal samples than sedimentation technique. Furthermore, the study revealed that gastrointestinal helminth parasites are more widespread in cattle.

**Indre *et al*., (2011)** reported that study was to identify to *Trichostrongyles* species and other nematodes parasites in sheep in Timis County. They worked on necropsy on 15 sheep, from which processes of gastrointestinal masses. Identification and harvesting of adult were used by slandered keys described by Soulsby (1965) and Dunn (1978). Identified species were *Ostertagia trifurcata, Teladorsagia circumcinta, Trichostrongylus colubriformis, Haemonchus contortus, Nematodirus filicollis, N. spathiger, Cooperia curticei* and *Bunostomum trigonocephalum.*

**Lone *et al*., (2012)** examined the prevalence of gastrointestinal nematode parasitic infections in goats (*Capra hircus*) of Baramullah District of Kashmir Valley. *Haemochus contortus* was found to be most prevalent parasites in host as it showed prevalence of 60% infection as compared to *Trichuris ovis* (51%), *Oesophagostomum* sp. (45%) and *Chabertia* spp. (1%).

**Kuchai *et al*., (2012)** in the present study made an attempt was made to find out the various nematodes and their prevalence infesting the pashmina goats of Ladakh through faecal examinations Identification on the basis of various morphological and morphometric characters Of the 70 animals examined 22 (31.42%) were found infected with single or multiple parasite species. Female goats were more infected than the males (37.14% and 25.71%). kids were more infected as compared to the adult (34.28% and 28.57%).

**Nuruzzaman *et al*., (2012)** carried out a study on 250 gastrointestinal tracts collect from different regions of study area and examined according to standard protocol with respect of prevalence, species composition and worm burden of goats during November 2009 to April 2010. Two species was identified which collected from abomasums of goat. Overall prevalence were 74.00% ($n$ = 250) and most prevalent species was *Haemonchus contortus* (58.00%) as campared to *Trichostrongylus axie* (16.00%). They also observed that infestation of abomasal nematodes parasites with the help of gender, age, breed and nutritional status, respectevly. Statistically data was showed that no significant difference observed the infestation of abomasal nematodes parasites in host in respect of the parasitic prevalence and worm count of two nematode parasites. The result showed that higher worm burden in *Haemochus contortus* (6.02±0.0928) and lower in

*Trichostrongylus axie* (0.04±0.14) in per animal. Geographical conditions also increased due to the parasitic infection of *Haemochus contortus* in research area.

**Nizam *et al.*, (2013)** gastrointestinal parasitism represents harsh health problems in small ruminant animals. The fresh faecal samples were collected from the gastrointestinal tract of goats. Total 340 goats were examined through faecal and tract examination for helminth infections. Helminth parasites were reported *Haemonchus* sp., *Ostertagia* sp., *Bunostomum* sp., *Oesophagostomum* sp., *Trichuris* sp., *Nematodirus* sp., *Paramphistomum* sp., *Fasciola* sp., *Dicrocoelium* sp., *Moneizia* sp., *Stilesia* sp., and *Avitellina* sp. (14.11%, 21.47%, 8.23%, 11.17%, 10.0%, 23.82%, 35.88%, 30.0%, 32.94%, 37.94%, 47.94% and 18.23%). Season wise study was showed that, infection rate high in summer as compared to spring, autumn and winter (51.16%, 68.18%, 73.46% and 32.35%).

**Gebeyehu *et al.*, (2013)** reported the gastrointestinal parasitic prevalence in Korean native goats (*Capra hircusaegagrus*). Fecal samples were collected (241 goats in 57 herd) and examined for parasites and their eggs. Standard qualitative and quantitative techniques were used for identification of eggs parasites. Overall 69.3% goats infected,163 hosts were infected by coccidia, 54 infected by nematodes and 5 infecte by cestodes (67.6%, 22.4% and 2.1%) . *Eimeria spp.* was group of strongyle group (20.7%) was the most prevalent nematode detected. The *Eimeria* spp was most prevalent and significantly higher ($p<0.05$) than that of the other gastrointestinal parasites. Strongyloides (23.5%) was heaviest parasitic infected parasite as compared to other parasites. Mixed parasitic infections showed 31.7% infection rate in goats.

**Lamrioui *et al.*, (2013)** studied to investigate helminthes parasitic infection in goats. Species were identified as Trichostrongylus colubriformis, *Nematodirus spathiger*, *Teladorsagia circumcincta*, *Skrjabinema ovis*, *Trichuris ovis* and *Haemonchus contortus* overall prevalence of helminths was 79% (95/120). The most prevalent parasites were *Trichuris ovis* (62.5%) followed by *Trichuris colubriformis* (52.5%) and *Trichuris circumcincta* (48.33%). Helminth parasitic infection higher in autumnas compared to other season. Female hosts were more infected as compared to male hosts (73.6% and 86.6%).

**Zahran and Behiry (2014)** studied the prevalence, virulence characteristics and molecular diagnosis of *Salmonella* in young diarrheic cow and buffalo-calves. A total of 591 diarrheic cow and buffalo-calves as well as 55 say that normal cows and buffalo calves were subjected to bacteriological examination for isolation of *Salmonella* species. The result was that 59 *Salmonella* species out of 646 fecal samples representing a percentage of 9.75%. *Salmonella typhimurium* was the major species among the isolates (25), then *Salmonella anatum* (14), *Salmonella entritidis* (8), *Salmonella meleagridis* (7), *Salmonelladublin* (6) and *Salmonella infantis* (4).

**Owhoeli and Elele (2014)** studied a total 213 faecal samples from four households and abattoirs to confirm the parasitic prevalence of helminthes parasitic infections in exotic and indigenous goats in Port Harcourt, South-South, Nigeria. The study was carried out on 153 exotic goats (Red Sokoto) *Capra hircus*, 112 were parasitic infected with different species of helminths, in 60 indigenous breed of goats (West African dwarf) *Capra hircus*, 49 were also infected with various types of gastrointestinal helminths. This overall study 75.5% was recorded, out of which 57 (76.0%), 55 (70.5%), and 49 (81.6%) were identified for exotic goat in the months of May to September 2010, exotic goat in the months of October 2010 to February, 2011 indigenous goats, respectively ($p<0.05$). Species of helminthes exposed were, *Haemonchus, Strongyloides, Chabertia, Trichuris, Ostertagia, Bunostomum, Trichos trongyloida, Ascaris, Tenia, Avitelina, Fasciola, Eurytrema, Gastrothylax, Schistoso ma,* and *Dicrocoelium* spp.

**Brahma *et al.*, (2015)** examined the infestation of gastrointestinal helminths and intensity and abundance of gastrointestinal nematode parasitic (GIN) infestation in Black Bengal goat in West Bengal, India. A total of 40 goats in the age group of 3 months to 1 year were screened by Standard carpological technique for a period of one year (from Oct 2012 – September 2013). The overall parasitic prevalence of GIH infection was 71.04 %. Highest overall prevalence (80.63%) as well as intensity of GIN infection (402) through EPG (eggs per gram). *Haemonchus contortus* was most predominant species with overall parasitic prevalence (63.50 %).

**Blackie *et al.*, (2015)** in their study, observed the gastrointestinal nematode parasites infection in small ruminants in Ghana and the epidemiological factors influencing their prevalence. Tweleve nematode species belonging to 6 families have

been reported to infect these livestock in the country with Haemonchus *contortus* being the most prevalent helminth parasite in both animals. Epidemiological knowledge is critical to the development of a complete and sustainable strategy for controlling gastrointestinal nematode infestation in goats and goat in the different agroecological zones and management system in Ghana.

**Jan (2015)** reported the comparative analysis and epidemiological study in gastrointestinal helminths parasitic infections at District Peshawar, Pakistan during study periods (November 2012 to April 2013). Total 800 faecal samples were collected and examined which out of 442 samples were found positive in sheep. Egg identification and collection was used by Standard floatation techniques. Gender-wise parasitic prevalence showed 62% male and 66.5% female respectively. Nematode parasitic infection infestation was mainly prevalent (49.6%) as compared to trematodes (3.6%) and cestodes (2%). Male and female sheep were exposed the total of 10 species of gastrointestinal helminthes parasites with high abundance of nematodes parasites. Highest rate of infection was found in female sheep. The most abundant parasite was found to be *Haemonchus contortus*.

**Zvinorova (2016)** reported prevalence of gastrointestinal parasitic infections in goats. Total 580 gastrointestinal tracts were collected from Zimbabwe in the wet and dry seasons. Some methods were used for collection of eggs of ectoparasites and culture of larava (Larval packet test) of endoparasites. Parasitic prevalence was indicated of *Eimeria* oocysts (43%), strongyles (31%) and low infection of trematode and cestode. Season, sex, study area and age were significantly spreading of gastrointestinal infections ($p < 0.05$).

**Verma *et al.*, (2017)** reported that 86.11% are infected out of 1419 goats. Faecal matter was collected and confirmed parasitic infestations in goat during 9 months (2015 August to 2016 April). The parasitic infestations percentages were *coccidian spp* (71.45%), *strongyle spp*. (28.40%), *Moniezia* spp. (18.74%) and *Strongyloides* spp. (0.70%). Coccidia (81.07%) were most prevalent parasites in Jamunapari goats, although maximum strongyle prevalence was found(30.64%) in Barbari goats and highest Infection *Moniezia* spp. in Jakhrana goats. Age-wise, study showed that highest parasitic prevalence of coccidian in >6 to12 month age groups while higher prevalence of strongyles was showed in less 6 month age. 2 to 6 month

age group having more *Moniezia* spp. Infestation in 2 to 6 month age group study during September and October.

**Yasin *et al.*, (2017)** conducted a cross-sectional study and identified during November to June (2016-2017) in kurmuk woreda, Assosa Zone of Benishangul Gumuz Regional State, Western Ethiopia. Total no. of 384 faecal samples were collected through flotation techniques. Out of the total sampled small ruminants (384), 82 (21.35%) , were infested by Gastro intestinal nematodes. The current study showed that age-wise prevalence of nematodesinfestation showed no significant difference on both species. However, prevalence of nematodes infestation was significantly higher in young goat (23.8%) & sheep (33.3%) than adult goat (18.2%) & sheep (20.4%). The host species-wise analysis of the data didn't reveal statisticaly significant association (X2 = 1.55 & P > 0.05) with prevalence of GI nematodes infestation between both host species of small ruminants. However, higher prevalenceof GIT parasites were observed in sheep (26%) than in the goat (20%). The present investigation has revealedsignificant seasonal variation in the prevalence of nematodes infestation during the study period. In this regard, the prevalence of GIT nematode in wet season (24.9%) was higher than in dry season (17%). Thus, it depictssignificantly higher (P = 0.0004) prevalence of nematode parasitic infestation in both hosts during monsoon season compared to winter season. Gender-wise analysis of the data revealed highly significant differences ($p<0.05$) in prevalence of nematode parasitic infestation between male and female hosts in both species of small ruminants. In this study significant difference ($p<0.05$) was experiential in prevalence of nematode infection in parasites in relation to body condition score where a higher prevalence of parasites were recorded in poor and medium body conditioned host as compared to good body condition. Thus, the current study has explored association of parasitic infestation with various risk factors encompassing gender, age, season, body condition score (body weight) and host species. As documented in this study, nematode parasitic infections are one of the major problems that could hinder health and efficiency of host in the study zone.

**Suntaravitun *et al.*, (2018)** Total of 224 participants were included in study during period (June 2017 to August 2017) in many parts of Thailand, particularly in rural areas. Samples were collected and examined with help standard method.

Samples were staining with formalin ethyl acetate and smear method for Identification of intestinal parasitic. Overall prevalence was 16.1% in intestinal parasitic infections. helminth infections (14.3%) protozoan infections are more common in soil (1.8%). The nematode parasitic infection was (6.7%) followed by *S. stercoralis*, (5.0%), *A. lumbricoides* (1.3%) and *T. trichiura* (1.3%). *E. histolytica/dispar* (1.0%), *G. intestinalis* (0.4%), and *B. hominis* (0.4%) were the protozoans identified. Multivariate study showed a significant (*P*=0.020) association of intestinal parasitic infestation gender wise with the accustomed odds ratio of 2.4 and 95% assurance period (CI) of 1.1-5.2.

**Bandyopadhyay *et al.* (2009)** studied gastrointestinal parasite infestation which remained a permanent hurdle worldwide for livestock development. The main aim is rapid identification of parasites by taking suitable control measures. Adult nematodes were collected from the intestine of slaughtered of north eastern region of India. The sequence of second internal transcribed spacer (ITS-2) of rDNA (ribosomal deoxynucleic acid) of *Oesophagostomum columbianum* and *Oesophagostomum venulosum* (sheep and goat origin), a pair of specific primers for *O.columbianum* (OCspF/OCspR) and *O. venulosum* (OVspF/OVspR) were designed of primer.

**Ahmed *et al.* (2011)** reported gastrointestinal parasitism as the most serious constraint throughout the world in small ruminant which causes significant production loss in animals. Morphological Identification of gastrointestinal parasitic infection was done by microscopic method of worm and eggs. Molecular techniques were also used for the identification of different parasitic species as well as molecular level. These techniques mainly have been used to identify genetic diversity among parasite population. The degree of variation of the sequence should exit with in a spp. by various region including rDNA and mtDNA or repetitive DNA constituent, Which showed the significant variation in the number of repeat within individuals have been employed to achieve the identification of parasite species or strain.

**Hunt *et al.*, (2011)** reported and identified and quantified parasites, since 1977, across >2000 of the gastrointestinal parasites. These study was based DNA-based tests of *Trichinella* species are potentially zoonotic parasites which can be

spreading from animals to humans via undercooked meat. Ten publications describe DNA-based tests for the detection, identification and/or quantification of Trichinella species. There has also been some attention to delineating species and identifying strains using DNA markers (Rodriguez *et al.*,1995, Zarlenga and La Rosa, 2000, La Rosa *et al.*, 2003, and Zarlenga, 2005). As more tests become available for parasite characteristics that cannot be assayed in other ways, the attraction of DNA-based methodologies will probably increase. Persistence with research is needed if these goals are to be achieved.

**Lambery *et al.*, (2011)** studied molecular epidemiology was defined the application of molecular techniques to the dynamics of disease in a population. Direct examination of parasites from clinical or environmental samples genetic studies have also been used to determine the extent of genetic diversity of parasites spp. which diversity is associated with different host cycles or epidemiologically important phenotypes.

**Tavares *et al.*, (2011)** studied the parasitological, routine laboratory diagnosis involving conventional methods, such as optical microscopy, used for the morphological identification of parasites. Molecular biology techniques are increasingly used to diagnose parasite structures in order to enhance the identification and characterization of parasites. Molecular assays have comprehensively assisted in the diagnosis, treatment and epidemiological studies of parasitic diseases that affect people worldwide, helping to control parasitic disease mortality.

**Avares *et al.*, (2011)** studied on review of morphological and molecular identification of parasites. Molecular characterizations is modern technique used for diagnosis and confirm the parasitic infection in the parasite. There are many techniques usd for diagnosis of parasites like PCR (polymerase chain reaction), RT PCR (Real-time polymerase chain reaction, LAMP (Loop-mediated isothermal amplification), random amplified polymorphic DNA (RAPD), Luminex xMAP, amplified fragment length polymorphism (AFLP), and RFLP (restriction fragment length polymorphism) in addition to microsatellites. Molecular method is also used for treatment and epidemiological studies of parasitic diseases that is described for worldwide population of the host.

**Justine (2012)** indicated the parasitic infestation in host. Dignosis and identification of parasites with the help of morphological and molecular characterization of parasites. The helminths parasites (digeneans, larval cestodes, nematodes, acanthocephalans) were colleceted from gasterointestinal tract and gill of fish. Morphological detection of Fasciola hepatica was infection in Lymnaea viatrix and analysis by histological microtome. Molecular study of *F. hepatica* DNAmt amplification were showed that a 1200 bp of band and another of 1300 bp F. hepatica.

**Wang *et al*., (2012)** reported the gastestrointestinal parasites (*Bunostomum trigonocephalum and Bunostomum phlebotomum*) identified by the morphological and molecular from the sheep and cattle in Heilongjiang Province (China, and grouped genetically). The ITS-1, 5.8S, ITS-2, 18S and 28S rDNA sequences was amplified by polymerase chain reaction (PCR), the result were showing 381, 153, and 231 bp in length, and 392, 153, and 240 bp in length of cattle respectively,. After sequenced analysis compared with others sequence of the parasites by the GenBank, and then phylogenetic analysis showing relationships with parasites in sheep and cattle was 87.4%.

**San-Ke Yu et al., (2012)** revealed that morphological and molecular characterization of nematode parasites in Cashmere goat in Shaanxi province, China. ITS-1, 5.8S, ITS-2 rDNA, and 5' end of 28S rDNA was amplified of 6 nematodes parasites morphologically identified as *Oesophagostomum asperum* from goat. The ITS-1, 5.8S, and ITS-2 sequences of nematode parasites were 374, 153 and 259 bp in length, respectively. The ITS-1 and ITS-2 rDNA between O. asperum were interspecies difference with *Oesophagostomum spp.* and other parasites showed that *O. asperum* is closely related to *O. venulosum* respectvely.

**Roeber *et al*., (2013)** reported that parasitic infection is the major problem in livestock cause many infectiasias diseases and leading to many economical losses in the world. The current study was focused on to identify the parasitic species by the molecular method. Molecular methods have been commonly applied *in vivo* and *in vitro* techniques for the diagnosis of anthelmintic resistance parasites. Studies have confirmed that Real-Time PCR (RT PCR) and multiplexed tandem repeat PCR assays can replace the imprecise and time-consuming method of parasites.

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**Abramatov (2013)** reported that nucleotide sequences of the second internal transcribed spacer region (ITS-2) of rDNA in *H. contortus* and *H. placei* revealed six (2.6%) nucleotide differences between these two species. The intra specific difference in internal transcribed spacer region second in *Haemonchus* spp. was very low. ITS-2 sequences of *H. placeifrom* and *H. Contortus* were clearly and homology similarity in *Haemonchus* was also reported.

**Vanwyk and Mayhew (2013)** identified the larvae of the common nematodes of sheep, cattle and goats during the period of 2004. This study is based on morphological and micrometery measurements which was estimated of the lengths of the sheath tail, extensions of L3 stage of worm of all species who found in this area. For example, if the mean length of the sheath tail, sheath caudal and caudal tip of *Trichostrongylus colubriformis*, *Trichostrongylus axeii* and *Haemonchus contortus* are 2.0–2.7 in measurement.

**Tan *et al*., (2014)** reported Overall 118 gastrointestinal tracts were infected out of 416 in the Malaysia. This study has been identified of parasites collected from different animals like 11 tracts of cattles were infected (11.2%) out fo 98, deer 4 (5.7%) out of 70 (63.1%), goats 99 out of 157 and swine 4 (4.4%) out of 91. Parasites were identified as a *Trichostrongylus colubriformis* (75 (75.8% of 99) and *Trichostrongylus axei i*n 4 (4.0%) of 99 goats and 2 (50.0%) of 4 deer. Identification was done by microscopy positive. 93 samples was done by PCR method and sequencing of the Internal Transcribed Spacer II region (ITS 2). Specifically, HTC co-infections were more prevalent (71 or 45.2% of 157) as compared to HTA co-infections (3 or 1.9% of 157).

**Vadlejch *et al.*, (2014)** determined the prevalence of nematodes parasitic infection in sheep in the study area. Two species were identified by morphological and molecular method. Gender-wise prevalence showed that 90.2 % females and 84.2 % males were infected by *Haemonchus contortus* and 9.8 % females and 15.8 % males of *Haemonchus placei* were identified. In molecular based on ITS-2 region with FspBI endo-nuclease as well as through the sequencing analysis. The most conspicuous result of study was to indicate that they could not identify *Haemonchus spp*. at the species level.

**Sato (2014)** observed the infection of trichostrongylids which was major public health problem in Lao PDR. In this study, gastrointestinal nematodes parasitic infection were identified in accordance to intensity and abundence of infections in goats and cattle, where in animals greatly are used for meat production in Lahanam Village, Lao PDR. The total number of goats (23) and bovines (29) was collected and which have 14 goats and 11 bovines was infected in this area. 93% (13/14) of goats and 36% (3/11) of cattle were found infestation. Coproculture showed *Trichostrongylus s*pp. (goats 16%; bovines 48%), *Haemonchus* spp. (goats 69%; bovines 37%), *Cooperia* spp. (bovines 8%) and *Oesophagostomum* spp. (goats 15%; bovines 6%). After necropsy on goat, *Trichuris* spp. was also found. We confirmed the presence of *Oesophagostomum spp., H.contortus and T. colubriformis* by morphology and DNA sequencing analysis of the ITS region of rDNA.

**Santos (2014)** analysed the infection of Haemonchus contortus and Haemonchus placei species in the host by the Molecular and morphological methods. The total of 141 *H. contortus* and 89 *H. placei* adult worm were collected from infected lambs. Statistically data showed highly significant ($P<0.05$) results. Identified individually by PCR method with the help of with species-specific set of primer. However, measurements of parasites (*H. contortus* and *H. placei* ) based on tip of the larval tail and sheath tail length of the L3 stage of worm. 485 *H. placei* larvae (0.619%) had a sheath tail shorter than 85 μm, while 500 H. contortus larvae (0.8%) presented a sheath tail longer than 85 μm. The results indicated that 6.09% of the male adult specimens with 0.71% of infection in the host.

**Fatima *et al.*, (2015)** described the prevalence of *Theileria lestoquardi* in small ruminants of Southern Punjab. A total of 115 samples from small ruminants

including goat (66%) and sheep (49%) were collected from five sampling sites (Bahawalnagar, Dera Ghazi Khan, Layyah, Multan and Muzaffargarh districts) in Southern Punjaba (Pakistan). Prevalence studies showed that *T. lestoquardi* was recorded to be 3.47% (n=4) through PCR amplification of their *18S rRNA* gene that produced a 730 bp DNA fragment. Highly significant (P = 0.00003) risk factor of parasites in small ruminants was observed.

**Meshgi (2015)** reported *Haemonchus* sp. was the most prevalent parasites which is leads to a very negative effect on the farming industry throughout the world, especially in the tropic and sub-tropic countries. The identification of parasites by morphometrics of the spicules and molecular characters. A total of the 270 adult male nematodes were collected from the abomasums of different ruminants (90 samples from each animal) at the different slaughter houses from different areas in Iran. Total 10 samples were used to isolate the DNA of *Haemonchus* spp. PCR-restriction fragment length polymorphism (PCR-RFLP) assay of the ITS2 of ribosomal DNA were described to confirm 1 (278 bp) and 2 (113 and 135 bp) different fragments, respectively. Moreover, concerning the ITS2-rDNA, sequences of 295 bp and 314 bp were obtained from *H. contortus* and *H. longistipes*, respectively.

**Fatima and Saeed (2015)** studied the prevalence of *Theileria lestoquardi* in small ruminants in Southern Punjab and conclude risk factors of parasitic infection. Prevalence of the *T. lestoquardi* was recorded to be 3.47% through PCR (polymerase chain reaction) amplification of 18S rRNA gene that produced a 730 base pairs DNA fragment. Blood samples of sheep collected from Multan indicating that sheep were more prone to this parasite (P = 0.03). It was experiential showed that tick parasitic infection was highly significant (P = 0.00003) in the host and also risk factor associated with the *theileriosis* in small ruminants.

**Gaherwal (2016)** reported that prevalence and infestation of nematodes in goats at 5 different villages, and district of Barwani (M.P.). A total 250 host, 50 goats was analyzed from each village. Worm and eggs were collected from different region of study area like, Khetia, Pansemal, Barwani, Sendwa during rainy, winter and summer season and Niwali. During the study 5 genera of nematode parasites were identified like. *Haemonchus spp., Trichuris spp., Nematodirus spp., Trichostrongylus*

*spp., and Strongyloides spp*. The overall eggs infection during rainy season was 76.8%, 72% during winter season and 66% in summer season. Parasitic infection was showed that 78% in rainy season, 72.4% in winter season and 66% in summer season.

**Hasheminasab *et al.*, (2016)** studied the nematode parasites (Parabronema skrjabiniis) of the family Habronematidae that parasites lives in the abomasums of ruminants such as sheep and goats. The study investigated the morphological and molecular identification of *Parabronema skrjabiniin* in sheep and goats from different regions of Iran. Morphological identifications were based on spicules of male, the frontal shield, egg dimensions and body length. The length of spicules of parasites were 657.5 μm and 304.07 μm and in goats 653.08 μm and 302.66 μm, respectively. Average morphological study showed that length across gender (male (16.5mm) and female (36mm) in sheep and (male (16mm) and female (35.5mm) in goats, respectively. PCR amplification was used two to specific primer (Para-Ir-R and Para-Ir-F) based on ITS2 (internal transcribed spacer 2 ribosomal) DNA marker (ITS2-rDNA) for identification of *Parabronema skrjabini*. The amplified products were found between 167 and 299 bp in different isolates (ITS2-rDNA sequences). Homology was showed between 68 % and 77% compared with another sequence data from GenBank.

**Zainab and Khan (2016)** studied the gastrointestinal parasitic infection in goats infected by morphological and histopathological study. Anterior end in mucosa, surrounded with fibroblast and leucocytes in histopathological sections of parasites. No sign of fibrosis and necrosis was found. Crypts of Leiberkuhn, submucosa muscularis mucosa and muscularis externa were evidently differentiated with minor permeation of provocative cells like mononuclear cells, leucocytes, goblet cells and along with epithelial changes and overall mucosal architecture in vicinity of worm.Prevalence rates of infection between 1% and 5.43% in sheep, 0.8% in wild sheep, 28% in camel and 1.72% in buffalo in Iran.

**Hassan *et al.*, (2017)** studied a total of 100 positive faecal samples which were collected and examined by method of floatation technique to detect strongylide eggs. Prevalence was recorded *Haemonchus contortus* (47%), *Trichostrongylus spp* (4%), *Oesophagostomum columbianum* (0%) and mixed infection (3%). Faecal samples were stored at frozen in -20°C and DNA extraction by using P:C:I

(phenol/chloroform/isoamylalcohol) protocol. Synthesized primer was used and run the PCR and found PCR pproduct. PCR products were amplified the region of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA of strongylide species.

**Ghasemikhah (2017)** studied the nematode parasites (*Strongyloides stercoralisis)* causing serious infections in immune compromised patients. Faecal sample was collected and the DNA was extracted and PCR-based method targeting the internal transcribed sequence 2 (ITS2) of the ribosomal DNA region was examined for the molecular detection of *Strongyloides stercoralisis* infection. Out of total 1800 patients only 5 stool samples were found positive infected with *Strongyloides stercoralisis* by the PCR method.

**Singh *et al*., (2018)** studied the dependence on agriculture and livestock of their marginal people and livelihood and found that 6.38 lakh villages and 72.2% population of India are dependent. Households cultivating >2.0 ha of land (small/marginal) was the warden of <76% of the total goats in the country. Goat has been playing important role in livelihood for the rural people by providing income, nutrition, employment, supporting crop production and risk repugnance in case of crop loss. Landless men and women are more and more relying on goat observance for their socio-economic upliftment.

# CHAPTER-3

# To Study the prevalence of gastrointestinal nematode parasites in goats at Lucknow

# CHAPTER–3
# TO STUDY THE PREVALENCE OF GASTROINTESTINAL NEMATODE PARASITES IN GOATS AT LUCKNOW

## 3.1 Introduction

Goat rearing is an important part of livestock, which is economically important to the farmers and also contributes for the national and international economy. This plays an important role in production of meat, skin, milk and organic fertilizer and also important in contribution of foreign exchange by exporting leather, and other products made from horns, bones, and teeth (Alam, 1993). The small ruminants have benefited little from vast resource owning to an assembly of difficulties, diseases are most dangers for the health of goats (Demelash *et al*, 2006). The major constraint which leads to large economic losses attributable to reduced weight gain, retarded growth and decreased production of goat farming (Jas and Ghosh, 2009).

Goat meat production in study areas due to prevalence of helminthes parasites is leading to high mortality and economic losses (Al-Quaisy *et al.,* 1987). Helminths parasitic infestation is major problem to both animals and humanbeings (Colley, 2001). Cestode, nematode and trematode are three classes of the helminth. Nematodes are most prevalent parasites in this study area. They are responsible for loss of productivity and caused many diseases like anoxeria, gastrointeritis, abdominal distention, diarrhoea, emaciation, bottle jow, affecting on meat production (Urquhart *et al.*, 1987) and serious economic losses to small and marginal farmers and the nation (Junaidu, 1997). Some major helminth parasitic infections were found to be prevalent in gastrointestinal tract of goats (*Haemonchus* sp., *Trichuris* sp., *Oesophagostomum* sp., *Bunostomum* sp. *(*nematode*) Moninezia* sp. (cestode) and *Paramphistomum* sp. (trematode).

Climatic conditions are responsible for the parasitic infection for the development, survival and spreading of nematode parasites, they are confirmed in many studies (Odoi *et al.*, 2007). They provide suitable environmental conditions for the transmission of parasitic infection in the host. (Mohanta *et al.,* 2007,

Varadharajan and Vijayalakshmi, 2015). Climatic changes have been implicated as a dynamic force for parasites, and made relationship between host and parasitic interaction (Naomi 2012).The prevalence of gastrointestinal nematode parasites in goats are dependent on quantity and quality of pasture managment, rainfall, temperature (maximum and minimum), humidity (maximum and minimum) and season (summer monsoon and winters) (Pal, 1993, Mitchell *et al.,* 2005 and Dijk *et al.,* 2008, Kedarkarki *et al.,* 2012 and Raza *et al.,* 2009 and Rahman, 1994). Meteorological Observation has been used to find the climatology of rainfall, temperature, humidity and wind in the study area (Singh *et al.,* 2012). The present study investigated the major gastrointestinal nematode parasites to assess the major risk factors associated with infection in goats.

## 3.2 Materials and Methods

### 3.2.1 Study area

For the present research, Lucknow (Uttar Pradesh) having five zones i.e., North, East, West, South and Central zone was selected for the prevalence studies of gastrointestinal parasites of goat. Details of areas are depicted in Table 3.1 and Figure 3.1.

**Table 3.1 Zone-wise study areas in Lucknow (Uttar Pradesh)**

| S. no. | Zone | Areas selected. |
|---|---|---|
| **1** | East | Gosaeganj, Gagaganj, Kabirpur, Moajjampnagar |
| **2** | West | Rajajipuram, Dubagga, Rajendra Nagar, Alamnagar |
| **3** | North | Baglabajar, Alambagh, Charbagh, Nakhhas, Tiwaripur |
| **4** | South | Mohalalganj, Uthretia, Vrindavan, Telibagh, Bhadruk, |
| **5** | Central | Rajnikhand, Orangabaad, Gauribajar, |

### 3.2.2 Study duration

The study was conducted from May, 2016 to April, 2019 in Lucknow district, located in the northern part of Uttar Pradesh.

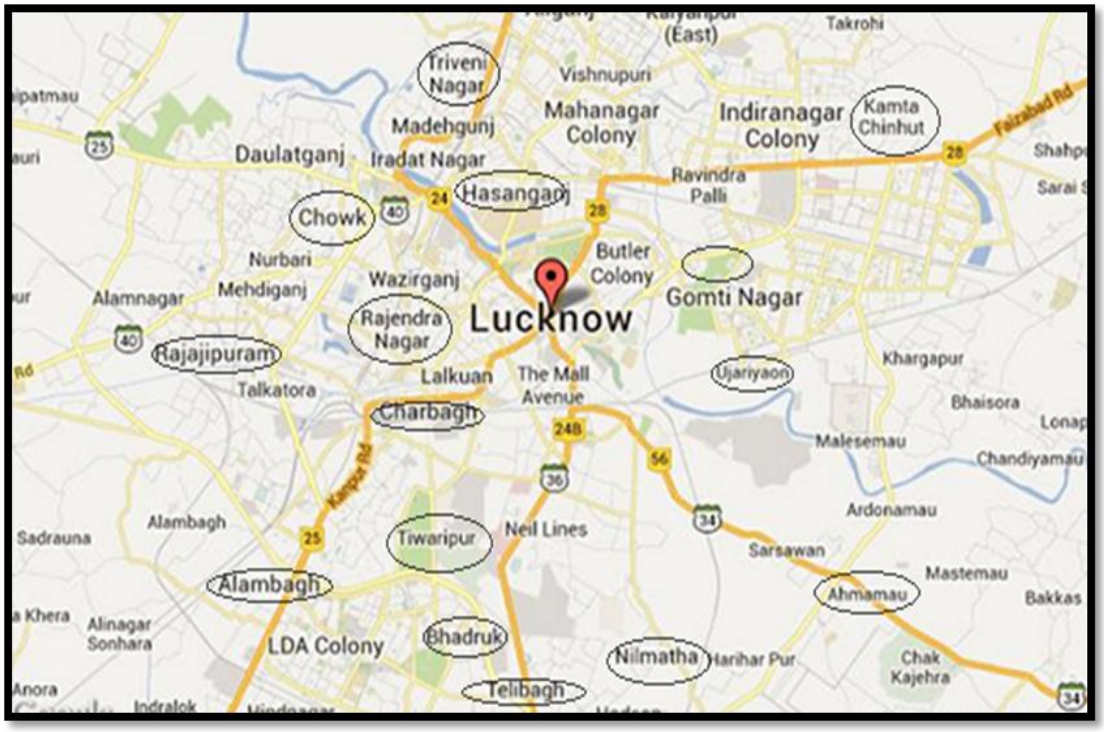



**Figure 3.1 Location Map of Study Area: Uttar Pradesh (*Source: Census of India, 2011*).**

### 3.2.3 Climate

The climatic condition of this study area is mainly depending on monthly rainfall, ambient temperature, humidity and these data were obtained from the National Meteorological Services Agency at Lucknow district, Uttar Pradesh (India). Geographical study area is located on 26.50° N and 80.50° E. Seasonal variation is depending on temperature, humidity and rainfall. Monsoon season having an average rainfall of 1010 mm (40 in) in Lucknow, June and July are the hottest months (av. max. and mini. temp. of 40°C and 12 °C temp., respectively) whereas December and January are the coldest months (av. max. and mini. temp. of 12°C and 4°C, respectively). Whole study was conducted in three seasons i.e., summer (March to June), monsoon (July to October) and winter (November to February) in three years (2016 to 2019).

### 3.2.4 Host

Goat is the most rearing animal in this area. Goat farming is very suitable for the small and marginal formers compared to other livestock. Furthermore, goats can be reared under limited resources and relatively small area of pasture. Goat breeds inclusive of Barbari, beetal, and Jamunapari breed were taken as sample hosts for studying the prevalence of parasitic infestation.

**Table 3.2** Scientific classification of goat

| **Kingdom** | **Animalia** |
|---|---|
| Phylum | Chordata |
| Class | Mammalia |
| Order | Artiodactyla |
| Family | Bovidae |
| Subfamily | Caprinae |
| Genus | Capra |

### 3.2.5 Host Age

The age of the host was confirmed by examining host's teeth and counting the rings of horn (Rahman and Hossain, 1997). Age of the host was categorized into three groups.

- Group I: <6 months
- Group II: betweeen 6-12 months
- Group III: >12 months

### 3.2.6 Collection and Preservation of Parasites

The gastrointestinal tracts of host were collected from different regions of Lucknow and brought to the Zoology lab of Babasaheb Bhimrao Ambedkar University, Lucknow for detection of parasites. Goats were categorized based on the

age, sex and breed which were carefully examined for the presence of parasitic infection.

Parasites were collected, cleaned with saline water or lukewarm water and then were transferred into 70% hot alcohol for 24hrs and then transferred to glycerol and alcohol (3:1).

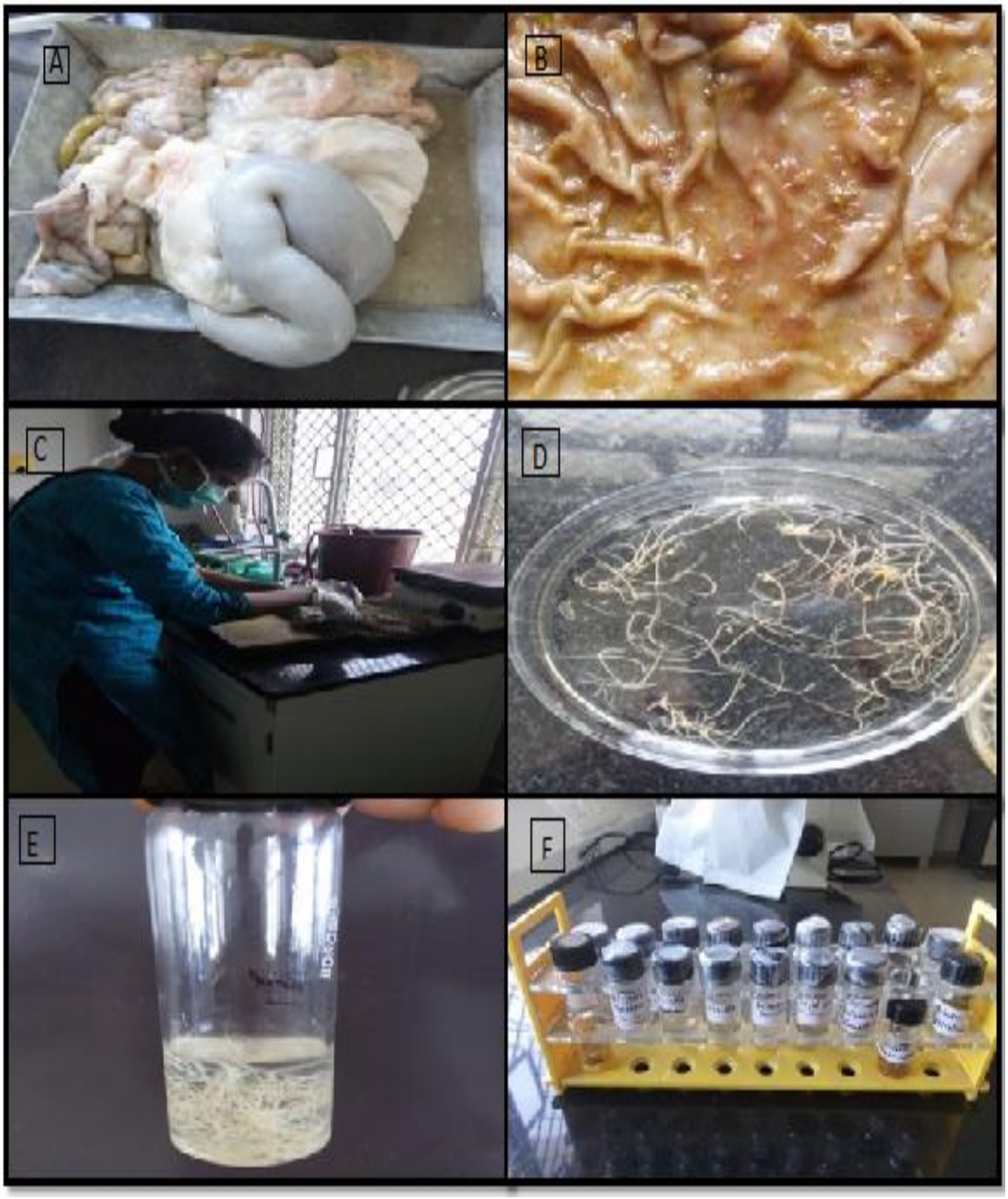



**Figure 3.2 (A) Gastrointestinal tract (B) Dissected gastrointestinal tract (C) Image during dissection (D) Collected parasites (E) Dehydration of parasites (F) Preservation of parasites**

### 3.2.7 Identification of eggs

Faecal samples were also collected from gastrointestinal tract of goat for identification of eggs. Collected sample was examined for the presence of egg with the help of sedimentation and flotation techniques (Perry and Hasson, 1984 and Soulsby, 1982). Samples were stained using methylene blue and wetmount solution. Prepared slides were observed under microscope (10X and 40X). Eggs of different nematode parasites of goats were identified on the basis of morphological appearance and size. Parasites were mounted in glycerol and images were taken under the light microscope (10x and 40x).

### 3.2.8 Statistical Analysis

Data was analyzed using Chi-square with the help of SPSS software version -20. The parasitic infections were calculated using the formula (Margolis *et al*., 1982).

$$\text{Prevalence (\%)} = \frac{\text{Total number of indivisuals infected with a perticular parasite species}}{\text{Total number of hosts examined}} \times 100$$

$$\text{Mean Intensity} = \frac{\text{Total number of individuals of a particular parasite species}}{\text{Total number of infected individuals of the host species}}$$

$$\text{Abundance} = \frac{\text{Total number of individuals of a particular parasite species}}{\text{Total number of the host species examined (infected + uninfected)}}$$

$$\text{Eggs/g faeces} = \frac{\text{Total number of eggs counted}}{\text{Total amount (in g) of faeces examined}}$$

### 3.2.8 Statistical Analysis

Data was analyzed using Chi-square with the help of SPSS software version -20 and Microsoft office excel. The parasitic infections were calculated using the formula (Margolis *et al*., 1982).

## 3.3 RESULTS AND DISCUSSION

The study revealed the epidemiological data of gastrointestinal helminth parasitic infection in goats across various parameters during the study period of May 2016 to April 2019. The data was collected from different regions of Lucknow (Uttar Pradesh, India).

### 3.3.1 Prevalence of gastrointestinal parasitic infection

The overall prevalence of gastrointestinal parasites of goats were found to be 63.33% out of total 540, during the 3 years (May, 2016 to April 2019), collected from the gastrointestinal tracts of goat. During the study period, it was found that 369 gastrointestinal tracts were infected and 117 tracts were non-infected ($X^2$ =.157, and p>0.05). Overall prevalence of parasites in goat is depicted in table 3.3 and figure 3.3. Similar studies findings many researches like, Akhtar *et al*., (2011); Raza *et al*., (2007); Mbaria *et al*., (1995); Maingi *et al*., (2001); Nwosu *et al*., (2007); Bandyopadhyay *et al*., (2010); Hassan *et al*., (2011) and Hansen and Perry (1994). The prevalence of gastrointestinal parasites infection was higher in goats than other ruminant animals, it may be due to lack of proper immunity in the host against parasitic infection (Ngoka, 1983). Hashem (1997); Arafa *et al*., (2007) and Ibrahim *et al*., (2008) also reported that management systems also has an impact on infection increases in the overall rate of gastrointestinal parasitic infection. This may be due to favorable conditions available for the growth of parasites and development of infective worm (Wisdom *et al.*, 2014). Similar studies were also reported from different regions of Hydrabad, (Akhtar *et al*., in 2011), Southern Punjab (Mushtaq *et al*., 2011), Southern Punjab-Pakistan (Raza *et al*., 2007). Researchers across the world also reported similar results, Kenya (Mbaria *et al*., 1995), Kashmir (Makhdoomi *et al*., 1995), Kenya (Meingi *et al*. 2001), Nigeria (Nwosu *et al*., 2007), India (Bandyopadhyay *et al.*, 2010), Bangladesh (Hassan *et al*., 2011) and Ethiopia (Tefera, *et al*., 2011 and Nabi, 2013).

**Table 3.3 Overall prevalence of helminths parasites in goat**

| Host | Infected intestines | Non-infected intestines | Total (%) | Mean± SE |
|---|---|---|---|---|
| Goat | 369 | 171 | 540 (68.33%) | 360.0±106.61 |
| $X^2$=.157 (p>0.05) | | | | |

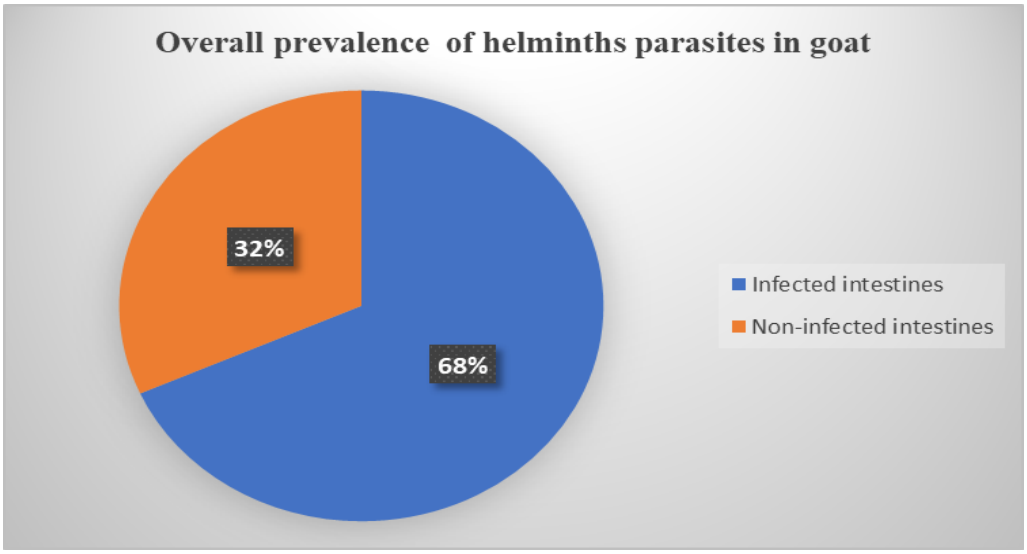



**Figure 3.3 Overall prevalence of gastrointestinal parasites**

### 3.3.2 Month-wise prevalence of gastrointestinal parasites

Month wise prevalence was studies and it observed that infection of helminth parasites in gastrointestinal tracts of goat during the study period. Higher prevalence of parasitic infection was found during July (94.11%) August (98.18%) and September (94.33 %) and lowest parasitic infection was found in months of January (20.00%), February (32.55%), March (58.69%) December (29.03%) (Table2). Similar findings were reported by many researchers like Nakanishi *et al.*, 2011, Shirale *et al.*, 2007, O'Connor *et al.*, 2007 and Skykes 1994. Statistically data was revealed that significant difference between prevalence during all months, ($X^2$, 146.94 and $p<0.05$). Similar studies were conducted by Rahman *et al.*, (2017) and reported. They examined the 270/426 infection in Bangladesh and found infestation rates of 63.4%. Others researcher like Grace Oyiza, Anibasa University of Abuja, 2014 (53.64%) also has reported sevrer parasitic infection in goat. Singh *et al.*, in Jabalpur (India) 2015 and Shaibani *et al.*, (2008) in Hyderabad reported that the poor pasture management was increased the parasitic infectivity in August and lowered in

January and February. Other studies were also reported in different parts of the country including Central Ethiopia (16.4%) (Bekele *et al*., 1992), Southern Ethiopia (98.9%) (Amenu, 2005) and far region (55.0%, sheep and 22.5%, goats) (Dereje, 2008). These results are consensus that the prevalence of parasites from different region, corresponding to climatic diversity as well as the accessible of host ranges (Njau *et al*., 1990 and Aga *et al*., 2003). Osakwe *et al*., 2007 and Nabi (2013) have reported the gastrointestinal parasitic prevalence in goat is caused by the poor management, lack of deworming program and illiteracy.

**Table 3.4 Month wise prevalence of gastrointestinal parasites**

| S. no. | Month | Total no. of intestines (2016 to 2019) | Infected intestines | Percentage (%) |
|---|---|---|---|---|
| **1** | January | 40 | 08 | 20.00 |
| **2** | February | 43 | 14 | 32.55 |
| **3** | March | 46 | 27 | 58.69 |
| **4** | April | 45 | 31 | 68.88 |
| **5** | May | 46 | 37 | 80.43 |
| **6** | June | 49 | 39 | 79.59 |
| **7** | July | 51 | 48 | 94.11 |
| **8** | August | 55 | 54 | 98.18 |
| **9** | September | 53 | 50 | 94.33 |
| **10** | October | 45 | 32 | 71.11 |
| **11** | November | 36 | 21 | 58.33 |
| **12** | December | 31 | 08 | 25.80 |
| **13** | Total | 369 | 540 | 68.33 |
| $X^2$= 146.94 ($p<0.05$) | | | | |

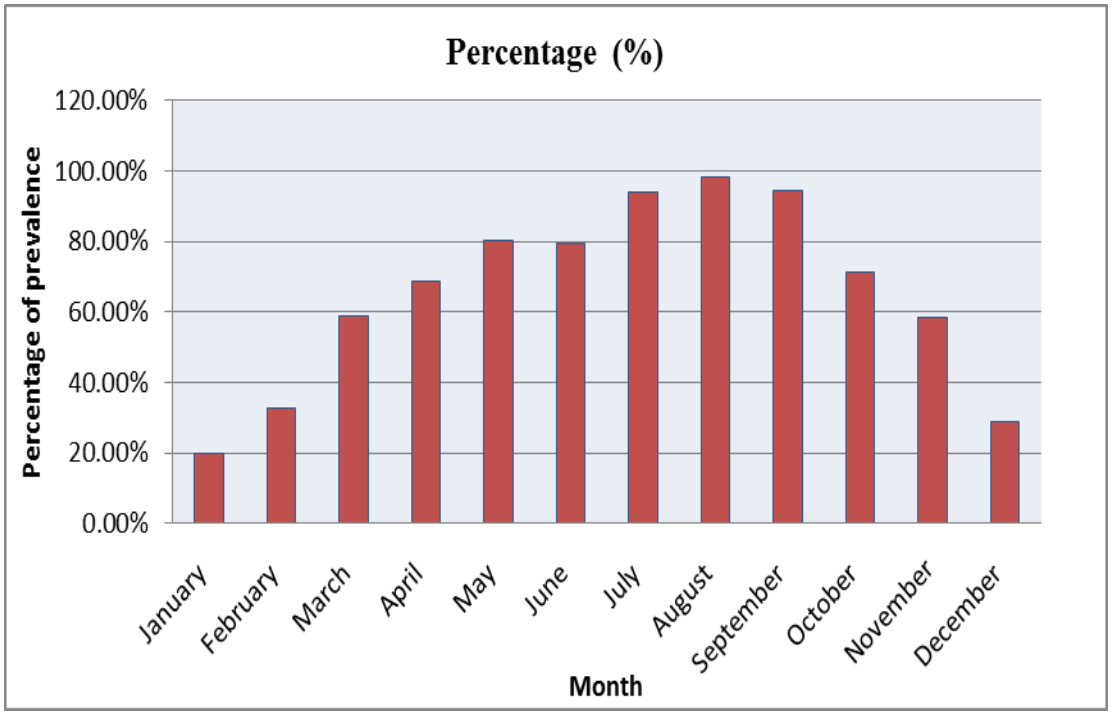



**Figure 3.4 Month wise prevalence of gastrointestinal parasites.**

### 3.3.3 Climatic factors

Prevalence of parasitic infections highely depends on different parameter like geographical distribution, climatic conditions and host factors which are required for the intensification of different stages of parasites (L1, L2 and L3). Geographical factors was also influences the parasitic prevalence in Lucknow in the state of Uttar Pradesh in India. The geographical location of Lucknow is between North (26.50°) and (East) 80.50°. Lucknow is located on elevation of 123 meters on top of sea the level.

Climatic factors depend on Meteorological data, which showed highest and lowest temperature and humidity, respectvely. The results also showed the (av. max and av. mini.) in the month of May (41°C. and 30°C) June (42°C and 33°C) and July (43°C and 35°C) and lowest temperature is showed in the months of December (25°C and 16°C) January (23°C and 16°C) and February (27°C and 15°C). Humidity also was evident (av. max and av. mini.) in the months of July (63% and 51%), August (71% and 50%) and September (63% and 52%) and lowest in the moth of March (27% and 22%), April (22% and 18%) and May (25% and 19%). Changes in climate factors might have direct effect on environment, pasture management

indicating those reason an increased or a decreased prevalence of the parasitic infection, abundance, and population dynamics (depending on the species) (Salem and López-Francos, 2017). Kao *et al*., 2000 and O'Connor *et al*., 2006 reported that temperature and humidity are responsible for parasitic infection. Optimal conditions increase the maximum number of worm eggs, and are difficult to predict. The range of optimum temperature for development also varies in a large range from av. 16 to 35°C for helminths, (O'Connor *et al*., 2006). L1 and L2 stage of worms survival in extreme temperature and aridness than L3 worm and L3 stage larva also survive in somedays in water at 3°C but they can be destroyed by freezing (Jasmer *et al*., 1987 and O'Connor *et al*., 2006). Similar studies were conducted by many researchers (Barger *et al*., 1972). Other researchers Kao *et al*., 2000; VanDijk *et al*., 2009 also have reported that climatic variability has been increased high L3 worm survival, like desiccation and ultraviolet irradiation increase mortality, but there are differences in susceptibility between parasite species.

However, some other workers like Roberts and Grenfell (1992) and Stromberg (1997) have reported the field of Europe, temperatures and humidity alongside, so aridity and ultraviolet irradiation estimating the persistence of parasitic infection in pastures. Armour (1986), Smith and Grenfell (1994) and Van Dijk *et al*., 2008 are also reported temperature variation effect on the parasitic prevalence. Variation results of the present and previous findings may be subjected to climatic factors, geographical conditions of the study area and methodology adopted for the study (Kabir *et al*., 2011).

**Table 3.5 Climatic factors (Temperature (°C) and Humidity (%) (Source: National Meteorological Services Agency at Lucknow district, Uttar Pradesh (India) 2016-2019)**

| Months | Infected intestine/ Total no. of Intestine (2016 to 2019) | Temperature (°C) | | Humidity (%) | |
|---|---|---|---|---|---|
| | | Max. | Min. | Max. | Min. |
| January | 08/40 | 23 | 16 | 53 | 45 |
| February | 14/43 | 27 | 15 | 42 | 25 |
| March | 27/46 | 32 | 20 | 27 | 22 |
| April | 31/45 | 39 | 27 | 22 | 18 |

| | | | | | |
|---|---|---|---|---|---|
| May | 36/46 | 41 | 30 | 25 | 19 |
| June | 39/49 | 42 | 33 | 34 | 22 |
| July | 42/51 | 43 | 33 | 63 | 51 |
| August | 53/55 | 34 | 35 | 71 | 50 |
| September | 51/53 | 33 | 25 | 63 | 52 |
| October | 32/45 | 34 | 24 | 47 | 26 |
| November | 21/36 | 30 | 20 | 37 | 25 |
| December | 07/31 | 25 | 16 | 39 | 28 |

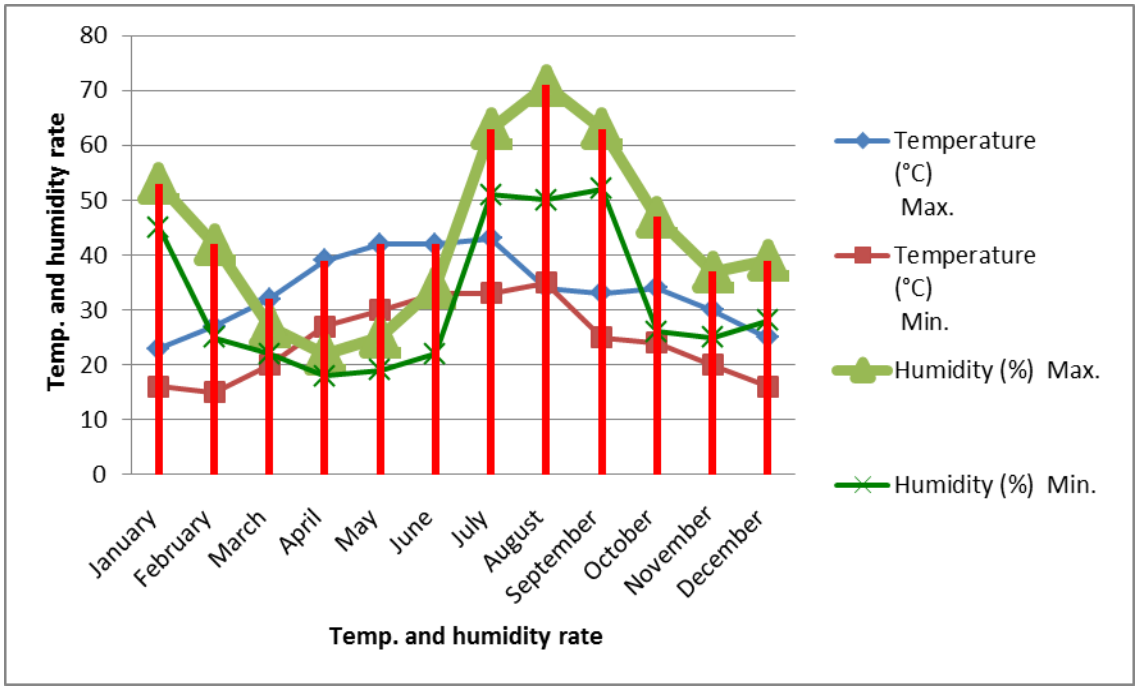



**Figure 3.5 Climatic factors (Temperature (°C) and Humidity (%) (Source: National Meteorological Services Agency at Lucknow district, Uttar Pradesh, India (2016 to 2019).**

### 3.3.4 Comparison study between nematode, trematode, cestode and mixed parasitic, prevalence

The prevalence of gastrointestinal helminth parasites in goat was very common. Goats were reported to be highly susceptible for different gastrointestinal parasitic infection. The present revealed was conducted to study prevalence of helminth parasites (nematode, trematode, cestode and mixed) month wise (January 2016 to December 2019). In the present study it was found that nematode parasites

were most prevalent in the months of July, August and September (53.13%, 58.18% and 54.71%) and lowest parasitic infection in January February and December (15.00%, 20.93% and 6.45%). Trematode infection was highly evident in the months of May, June aand August (8.69%, 12.24% and 09.09%) and lower in Febuary, March, April, November and December (2.32%, 6.52%, 2.22%. 2.77% and 3.22%). Cestode was recorded higher in August and September (11.32% and 20.00%) and lowers in months of January, February and December (2.5% and 2.77%). In this study, it was observed that Nematode parasites (37.77%) are most prevalent parasites compared to trematode (05.73%) and Cestode (7.03%) in the study area. Statistical analysis data was recorded ($X^2$ = 64.62 and $p<0.05$ Significant for nematode), ($X^2$ = 16.08 and $p>0.05$ Non-significant for trematode), ($X^2$ = 13.08, and $p>0.05$ Non-significant for cestode) and ($X^2$ = 17.76, and $p>0.05$ Non-significant for mixed infection).

Similar findings have been reported by Amran (2018), Ahmed *et al*., (2017), Getachew *et al*., (2013) and Mohammed *et al*., (2015). Kumsa *et al*., (2011); Zeryehun (2012) and Tigist *et al*., (2015) reported 53.29% and 83.1% gastrointestinal tracts were infected of goats in Central Ethiopia. The prevalence of helminths was also studied in several areas Kajiado District in Kenya (Nganga *et al*., 2004). Generally, young animals are more susceptible to nematode parasite infections. Nsereko *et al*., (2015) in this study in Uganda reported that older animals have developed better immunity against nematode infections (Tasawar *et al*., 2010). Various researchers also showed nematode higher infestation in different parts of the world (Ahmed and Ansari, 1987), Guiomaraes and Walter, 1987, Uriarte and Valderrabno, 1989, Pal and Qayyum, 1993 Opara *et al*., 2005). Ijaz *et al*., (2008) reported 13% in southeast Nigeria. Highest parasitic burden was reported by Abebe and Esayas (2001), Bersisa and Abebe (2006) (88.2%) and Sissay *et al*., (2007) 76.4% in goats. Nematodes were found to be the most prevalent than the other parasites and this might be due to geoclimatic conditions including climatic factors, optimal conditions (temperature and moisture), and nutrition affecting the immune systum and poor pasture and rearing pattern of the goats in the study areas (Alim *et al*., 2012).

**Table 3.6 Comparitive study between nematode, trematode, cestode and mixed parasitic, prevalence**

| Months | Infected Intestines | Total no. of intestines (2016 to 2019) | Nematode | Trematode | Cestode | Mixed |
|---|---|---|---|---|---|---|
| January | 08 | 40 | 06(15.00) | - | 01(02.05) | 01(02.5) |
| February | 14 | 43 | 09 (20.93) | 01 (02.32) | 01 (07.14) | 03 (06.93) |
| March | 27 | 46 | 13 (41.30) | 03 (06.52) | 05 (10.86) | 06 (13.04) |
| April | 31 | 45 | 24 (53.33) | 01 (02.22) | 02 (04.44) | 04 (8.88) |
| May | 37 | 46 | 23 (50.00) | 04 (08.69) | 02 (02.17) | 08 (17.39) |
| June | 39 | 49 | 20 (49.81) | 06 (12.24) | 03 (06.12) | 10 (20.40) |
| July | 48 | 51 | 26 (51.00) | 08 (15.68) | 05 (09.08) | 09 (17.64) |
| August | 54 | 55 | 35 (58.18) | 05 (09.09) | 03 (5.45) | 11 (23.63) |
| September | 50 | 53 | 28 (52.83) | 04 (07.54) | 06 (11.32) | 12 (22.64) |
| October | 32 | 45 | 16 (24.44) | 02(04.44) | 07(20.00) | 0 7(22.22) |
| November | 21 | 36 | 06 (16.66) | 01 (02.77) | 01 (02.77) | 13 (36.11) |
| December | 08 | 31 | 03 (09.67) | 01(03.22) | 02 (06.45) | 02(06.45) |
| Total | 369/540 | 540 | 206(38.14) | 36 (6.66) | 38 (7.03) | 86 (16.00) |
| | | | $X^2 = 64.62$ ($p<0.05$) | $X^2 = 16.08$ ($p>0.05$) | $X^2 = 13.08$ ($p>0.05$) | $X^2 = 17.76$ ($p>0.05$) |

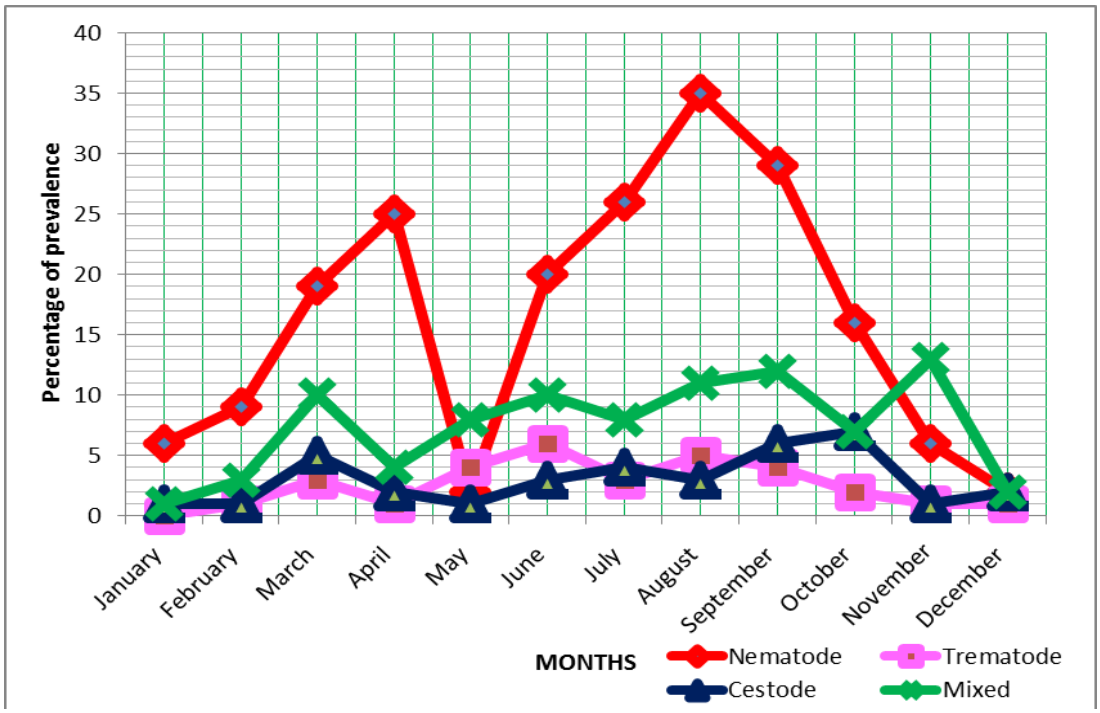



**Figure 3.6 Comparitive studies between nematode, trematode, cestode and mixed parasitic prevalence.**

### 3.3.5 Season-wise gastrointestinal helminth parasitic infection

In this study, seasonal prevalence of helminthes parasitic infection was highly significant ($X^2$=127.96 and p>0.05)**.** However higher infection rate was detected during summer and monsoon season (71.50%, 87.25%) but lower in winter (33.33%). Nematode infections were observed higher during the months of summer, monsoon and winters (44.08%, 50.98% and 16.00%), as compared to trematode (7.52%, 11.72%, and 4.00%), cestode (6.00%, 9.80% and 5.33%) and mixed infection (14.50%, 18.00% and 10.66%), respectively. However, variations in season-wise prevalence of parasitic infestation in goats was found significant ($X^2$=119.46, p<0.05 for summer, $X^2$=129.79, p<0.05 for monsoon and $X^2$ =20.81, p< 0.05 for winter). This seasonal effect has already been reported in previous studies (Sissay *et al.*, 2007 and Sutar *et al.*, 2010). The increase in their prevalence during summer may be due to increase in humidity and availability of favorable temperature. Heavy rainfall and humidity are inclined to heavy parasitic infection (Hawkins *et al.*, 1945).

Different climatic conditions also are prejudiced spreading of larvae which increased the possibility of contact between larvae and host (Croll, (1975) and

Soulsby, 1966). Islam *et al*., 2017 also reported findings that seasonal prevalence of gastrointestinal parasites was significantly higher ($p<0.05$) in rainy season (83.6%) followed by summer and winter ((78.6% and 59.4%) in Bangladesh. Almost similar findings were reported by Yadav *et al*., (2006) reported that highest parasitic prevalence in Jammu and Kashmir during monsoon season as comparison to summer and winter seasons (88.5%, 83.2% and 76.0%). Singh *et al*., (2015) reported most prevalent parasites during monsoon (98.0%) and lowest in winter (91.7%) in Madhya Pradesh, India. Singh *et al*., (2015) repored during mosoon (90.10%) highest parasitic infection, followed to winter (83.84%) and summer (78.35%) in Punjab, India. Moreover, Gebeyehu *et al*. (2013) reported from Korea and Talukdar (1996) from Assam (India). Higher prevalence during rainy season might be due to more favorable climatic conditions for development parasites of goat (Faizal *et al*., 1999 and Biswas *et al*., 2014).

Geographical and climatic conditions of the study area might be responsible for this variation of parasites. Lucknow is a subtropical region, so in this area is characterised with three seasons, summer, winter and rainy (Islam *et al*., 2017). Seasonal patterns of infection also show climate variation (Smith and Grenfell, 1994, Kao *et al*., 2000; O'Connor *et al*., 2006; Morgan, (2012) van Dijk, (2012) and Singh *et al*., (1997) reported that availability of parasitic infection on pasture was at its maximum during rainy season. The many studies showed high prevalence and intensity of gastrointestinal GI parasitic infection due to seasonal incidence in study area. (Skerman *et al.,* 1967; Uriarte *et al.,* 2003; Tariq *et al.,* 2008 and El-Azazy 1995). Muhammad *et al*., (2009) and Demissie (2013) reported that high humidity, microclimate of the faeces and the herbage is necessary and development and growth are also essential for survival for gastrointestinal parasites.

**Table 3.7 Season wise gastrointestinal helminths parasitic infection**

| S.no. | Season | Total no. of Intestines | Infected Intestines | % of prevalence |
|---|---|---|---|---|
| **1.** | Summer | 186 | 134 | 71.50 |
| **2.** | Monsoon | 204 | 184 | 87.25 |
| **3.** | Winter | 150 | 51 | 33.33 |
| **$X^2$=127.96 (>0.05)** | | | | |

**Table 3.7(a) Season wise comparative study of gastrointestinal helminths (nematode, trematode, cestode and mixed) parasitic infection**

| **Season** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Summer** | | | | **Monsoon** | | | | **Winter** | | | |
| Nematode | Trematode | Cestode | Mixed | Nematode | Trematode | Cestode | Mixed | Nematode | Trematode | Cestode | Mixed |
| 82 (44.08) | 14 (7.52) | 11 (6.00) | 27 (14.50) | 104 (50.98) | 24 (11.77) | 20 (9.80) | 36 (18.00) | 24 (16.00) | 06 (4.00) | 5 (3.33) | 16 (10.66) |
| **($X^2$) - 119.46 ($p<0.05$)** | | | | **$X^2$=129.79 ($p<0.05$)** | | | | **$X^2$=20.81 ($p<0.05$)** | | | |

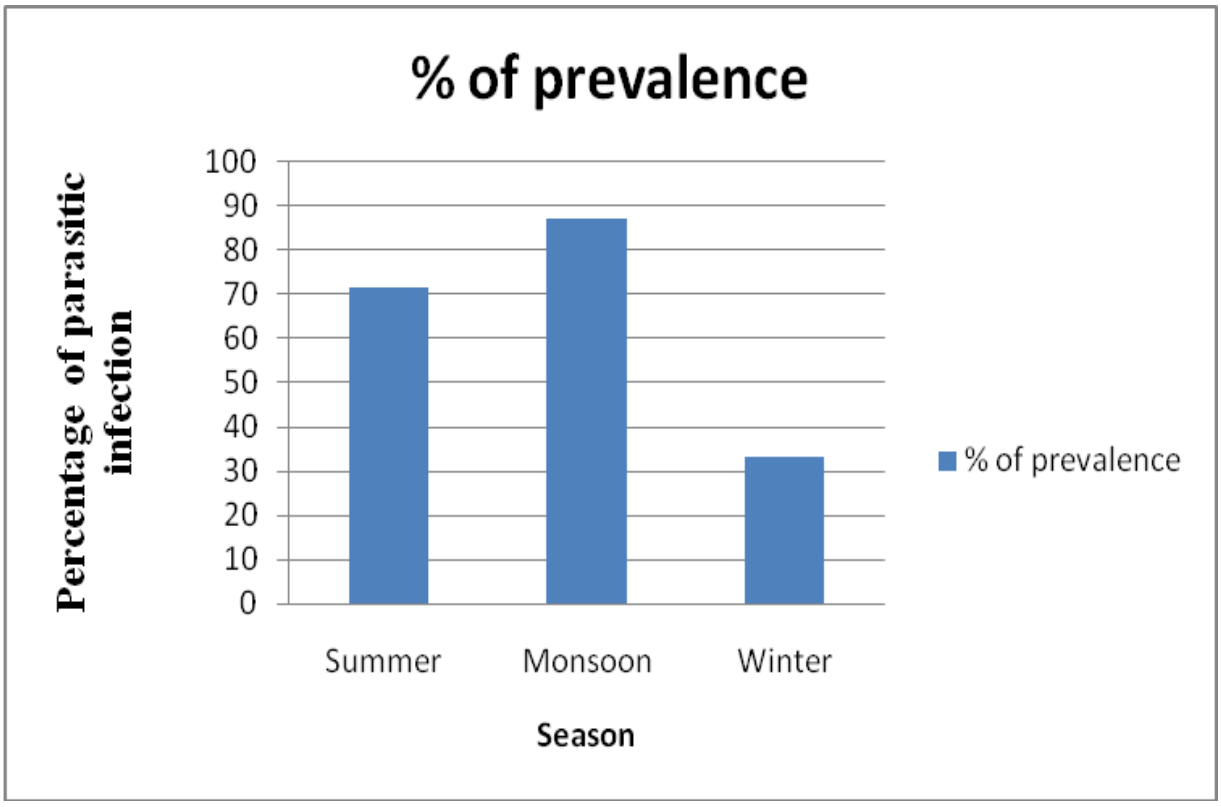



**Figure 3.7 Season wise gastrointestinal totle helminth parasitic infection**

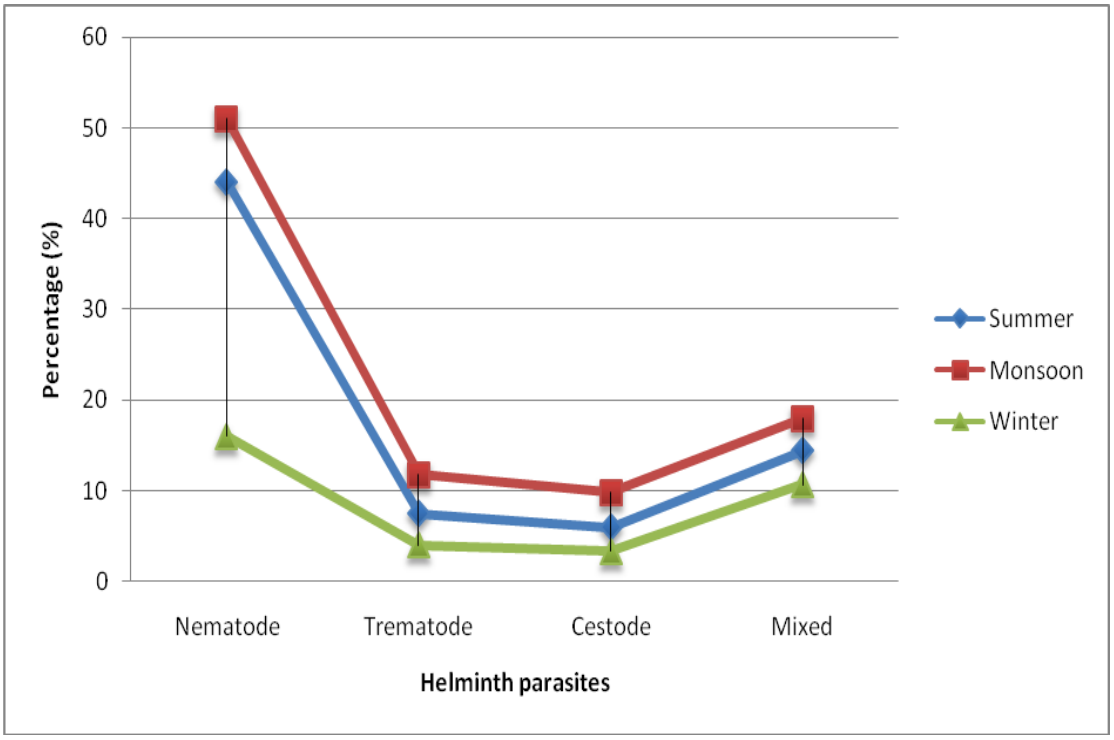



**Figure 3.7 (a) Season wise gastrointestinal nematode, trematode, and cestode (helminth) parasitic infection**

### 3.3.6 Species-wise helminths parasitic infection

The species-wise study revealed that gastrointestinal parasitic infection was reported in 369 (68.33%) goats out of 540 goats during 2016 to 2019 and statistically analysis showed nonsignificant data ($X^2$=300.34 and p>0.05). The identified gastrointestinal parasites were *Haemonchus sp.*, (376/440), *Oesophagostomum sp.*, (296/540) *Trichuris* sp., (246/540), *Paramphistomum* sp., (159/540) and *Moniezia* sp. (131/540). *Haemonchus* sp., (69.62 %) *Oesophagostomum* sp., (54.81%) *Trichuris* sp., (45.55 %) are nematode parasites, *Paramphistomum* sp. (29.44 %) is a trematode parasite and Moniezia sp. (24.25 %) is a cestode parasite. Nematodes are most prevalent parasites in this study area that study showed that *Haemonchus* sp. is the most dominant parasites as compared to other parasites. Highest mean Intensity is prevalent in the *Parasphistomum* sp. (19.01) and *Haemonchus* sp. (13.39) as compared to other parasites (*Oesophagostomum* sp. (09.55), *Trichuries* sp. (09.12) and *Moninezia* sp. (02.00) and Comparition study also showed highest abundance of parasitic infection in *Haemonchus* sp. (09.32), *Oesophagostomum* sp. (05.23)

*Paramphistomum* sp. (5.29) than in *Trichuries* sp. *(*04.15) and *Moninezia* sp. (0.10). People therefore should be aware and should be trained to minimize the risk. The parasite *Haemonchus* sp. parasite was found to be the most prevalent, similar findings was reported by Raza *et al*., 2014 in Cholistan desert of Pakistan. Katoch *et al*. 1999; Mbuh *et al*., 2008 and Raza *et al*., 2012, also reported similar findings. The higher prevalence can be credited the *Haemonchus* sp. lays eggs 10,000 to 15,000 eggs/day compare to other parasites. Many studies showed that *Haemonchus* sp. are most prevalent and also resistance against anthelmintics treatment and medicine so that these parasites grow faster and highly prevalent (Radostits *et al*., 1994 and Katoch *et al*., 1999). The abundance and intensity of nematodes infestation were higher over trematodes and cestodes parasites in the work reported by Kumsa *et al*., 2011; Raza *et al*., 2014 and Jan *et al*., 2015.

Parasitic infection also showed highest in *Haemonchus* sp. than the other parasites (*Oesophagostomum* sp., *Trichuries* sp., *Paramphistomum* sp. and *Moninezia* sp.). The difference in prevalence of nematode parasites is dependent on geographical, climatic factors, pasture management and strategies against husbandry (Nizam, 2013 in Kashmir and Dash, 2017 from Meghalaya). The species-wise studies were reported in many researchers Gadahi *et al*., (2009) and Dabasa *et al*. (2017) in Ethiopia and Pakistan, Islam and Taimur (2008) Gebeyehu *et al*., (2013) in Korea and Bangladesh; Hassan *et al*., (2011) found 63.4% prevalence of different species of parasites in the host. This variation might be due to the differences in geoclimatic conditions, rearing and pasture management of small ruminants along with nutritional status (Jugessur *et al*., 1998).

Bersissa and Abebe (2006) from Ogaden reported that Haemonchiasis was significantly higher than the other species in goats. Githigia *et al*., (2005) from Kenya and Wang *et al*., (2006) from China reported higher prevalence of *Haemonchus sp.* in abomasal parasites; it may be due to different factors like higher number of egg development. Getachew *et al*., (2007) suggested that *Haemonchus* sp. easily contaminate food in adverse climatic conditions through hypobiosis (Waller *et al*., 2004). Conder and Jonson (1996) reported a seasonal difference in nematode parasites occurs when humidity and temperatures increase, with significant rainfalls. These conditions are most favorable for the development and growth of different

stages of parasites. Similar studies were also done by VanWyk *et al*., (2004), where in it was reported that *Haemonchus* sp. is responsible for higher infection as compared to the other species (*Oesophagostomu* sp., *Trichuris* sp., *Paramphistomum* sp. and *Moninezia* sp.).

**Table 3.8 Species wise helminth parasitic infection**

| S.no. | Parasites | Infected intestines/Total no. intestines examined | Percentage (%) of infection | Total no. of parasites | Mean intensity | Relative Abundance |
|---|---|---|---|---|---|---|
| **1.** | *Haemonchus spp.* | 376/540 | 69.62 | 5036 | 13.39 | 09.32 |
| **2.** | *Oesophagostomum spp.* | 296/540 | 54.81 | 2829 | 09.55 | 05.23 |
| **3.** | *Trichuris spp.* | 246/540 | 45.55 | 2245 | 09.12 | 04.15 |
| **5.** | *Paramphistomum spp.* | 159/540 | 29.44 | 3023 | 19.01 | 5.59 |
| **6.** | *Moneizia spp.* | 131/540 | 24.25 | 262 | 02.00 | 0.10 |
| **$X^2$=300.34 (p>0.05)** | | | | | | |

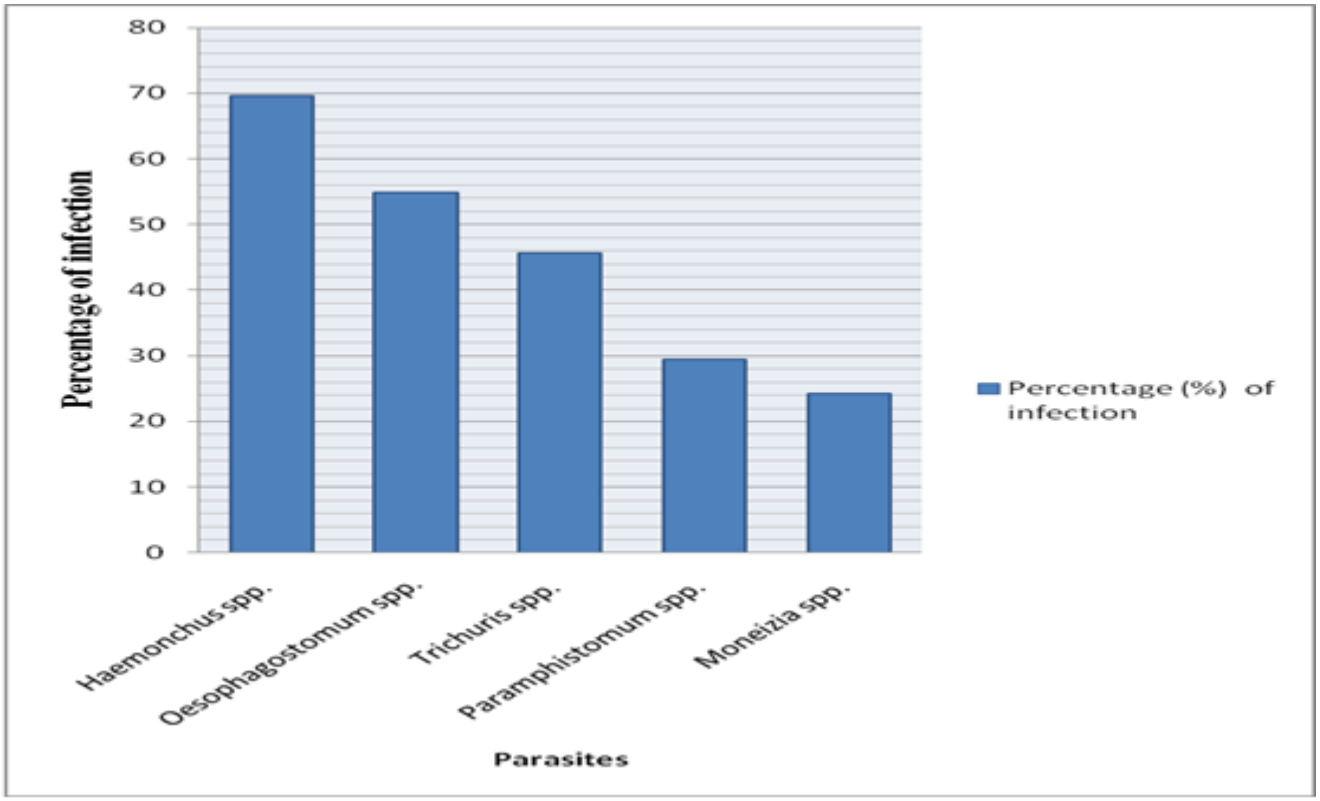



**Figure 3.8 Species wise helminth parasitic infection**

### 3.3.7 Age-wise helminths parasitic infection in goat

Age-wise parasitic prevalence revealed that the overall gastrointestinal parasitic infection was slightly observed in (> 6 month) Group-1 (74.48%), followed by 6 months to 12 month (Group-2) (57.14%) and <12 month (Group-3) (71.34%). In the present study, it was observed that Group-1 and Group-2 are very prone to gastrointestinal parasites infestation as compare to group-3 (Table no. 3.9 and figure 3.9). Highly significant differences in infected intestine on basis of age groups ($X^2$= 10.90 and p< 0.05)**.** Similar study has been reported by Shah *et al.,* (2015) and Das *et al.,* (2015). However, much difference in the prevalence of nematode parasites in kids and adults was not found and the findings are at par with the report of Singh *et al.,* (2015). Nizam (2013) also reported that kids have more infection and found to be more susceptibile for increasing the gastrointestinal helminth parasitic infestation in goats. Thus, the age is found to be factors are most important parameters in this study because immunity plays a significant role in the host body. Vlasoff *et al*., (2001) studied that, less resistance level of parasitism in young animals is due to the growth and development of significant immunity, which is to begin with low but increases with the duration of exposure intensity and abundance of parasitic infection.

The parasitic infestation was found to be higher in kids and adult than the young and this may be due to the reason that the kids and adult goats have acquired immunity to parasites through frequent challenges and expel the ingested parasites before they establish infections (Shah-Fischer *et al.*, 1989 and Dun, 1978). Laksmi *et al.,* (2001); Sissay *et al.,* (2006) Sharma *et al,* 2009; and Emiru *et al.* 2013, also reported that the kids are more susceptible as compared to adult and worm burdens of worm decrease with increasing age due to immunological maturity acquired after repeated exposure. Sangma *et al*., (2012); Raza *et al*., (2014) Poddar *et al*., (2017) also reported that higher parasitic prevalence in kids as compared to young animals in Pakistan. Singh *et al*. (2015) reported most prevalent in young (96.03%) compare than adults (93.9%) in India. Zvinorova *et al*., (2016) suggested that the higher prevalence in kids as host as compared to young and adult. But some researchers also noticed higher prevalence in young (group 2) in comparison with adults (group 3) in the animals (Anene *et al*., (1994), Uddin *et al*., (2006) and Hassan *et al*., (2011). Singh *et al*., (2015) also reported higher infection in kids because of low

immunity and higher susceptibility to the parasitic infection and lower parasitic prevalence among young and adults; it might be due good immunity and body resistance against to infection. (Bandyopadhyay *et al.*, 2009).

**Table 3.9 Age wise gastrointestinal helminth parasitic prevalence in year of 2016-2019.**

| S.NO. | Age (In months) | Infected Intestines/ Total no. of intestines (2016 to 2019) | Percentage (%) of Total infection |
|---|---|---|---|
| 1. | > 6 months (Group-1) | 109/145 | 75.17 |
| 2. | Between 6 months To 12 months (Group-2nd) | 131/ 217 | 60.36 |
| 3. | <12 months (Group 3rd) | 129/ 178 | 72.47 |
| **$X^2$= 10.90 (p< 0.05)** | | | |

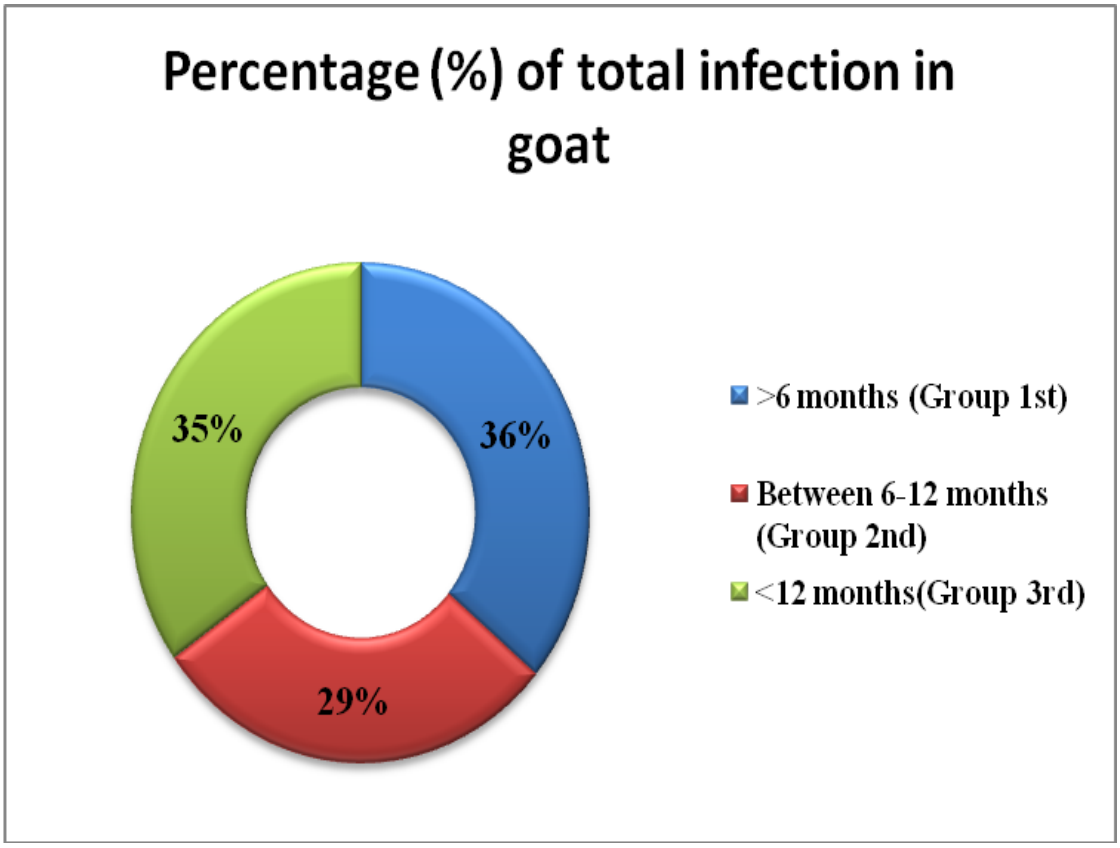



**Table 3.9 Age wise gastrointestinal helminth parasitic prevalence**

### 3.3.8 Sex-wise helminths parasitic infection in goat

In the present study, a total of 540 intestines inclusive of 293 female and 247 male goats were examined, out of which 208 female goat and 161 male cattle were found to be infested with gastrointestinal parasitic infection. The prevalence rate of parasites was found to be higher in female goats (70.98%) than the males (65.18%) (Table no. 3.10 and figure no.3.10). Non-significant differences in infected intestine on basis of sex-wise ($X^2$=2.09, p>0.05). The presence of sex difference in infection is also conflicted with other reports (Ghanem *et al.*, 2009 and Hassan *et al.*, 2013). In addition, Azrul *et al*., (2017) also reported that prevalence higher in female goats compare to male (75.42% and 56.72%) in Bangkok, (Thailand). Kabir *et al*., (2011) suggested that higher prevalence rate in female cattle may be due to hormonal effects. Sangma *et al*., (2012) and Yeasmin *et al*., (2015) in Bangladesh showed higher infection in female as compared to males. Similar study found in Singh *et al*., (2015) in Punjab and Kashmir, respectively. Higher prevalence of gastrointestinal parasites in females may be due to the stall feeding and stress during pregnancy, which is heavily infested due to hormonal differences and higher level of progesterone and prolactin hormones are also responsible for the increased the parasitic infection in females (Lloyd, 1983; Dagnachew,2011; Muluneh *et al.*, 2012 and Ayaz *et al.*, 2013). Mazid *et al*., (2006) also recorded 100% prevalence rate in the female host and 78.6% in male host. Dabasa *et al*., (2017) also reported high prevalence rate in female and this might be due to low immunity status during pregnancy, stress and lactation period.

**Table 3.10 Sex wise gastrointestinal helminths prevalence**

| S.No. | Sex | Infected intestines/ Total no. of intestines (2016 to 2019) | Percentage (%) |
|---|---|---|---|
| **1.** | Female | 210/540 | 56.91% |
| **2.** | Male | 159/540 | 43.08% |
| **$X^2$=2.09 (p>0.05)** | | | |

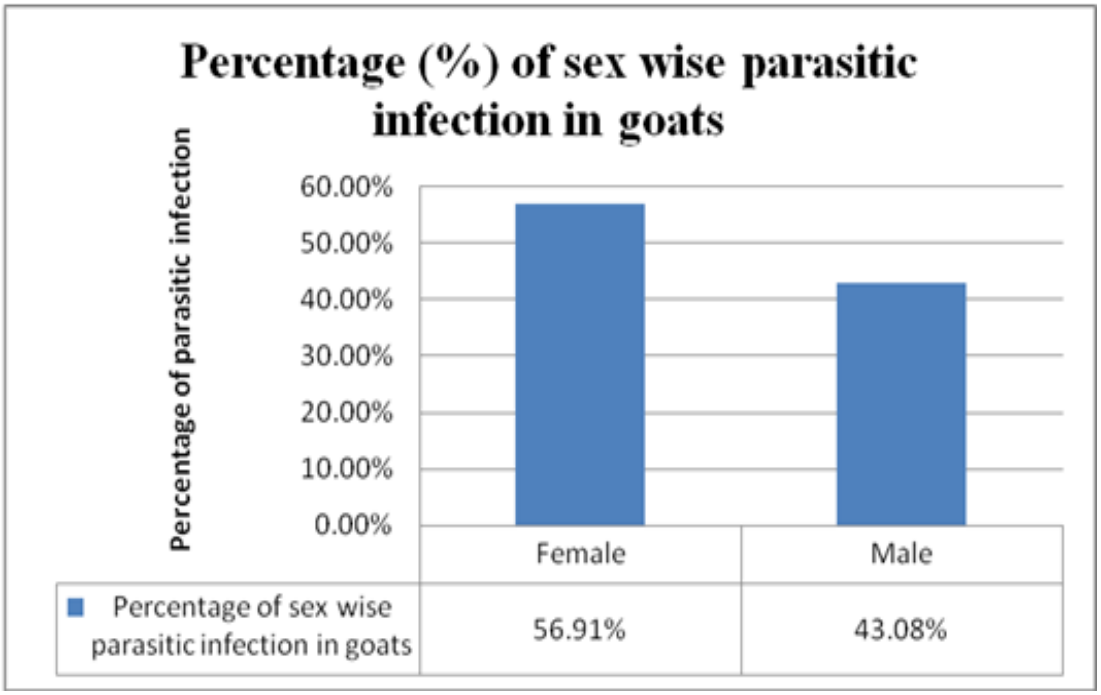



**Figure 3.10 Sex wise gastrointestinal helminths prevalence**

### 3.3.9 Body condition of goat

In this investigation the gastrointestinal parasitic infection is highly dependent on good (18.95%), moderate (28.14%) and poor body condition (40.18%) of the goat. In this study, it was observed that significant ($X^2$=18.62, p<0.05**)** prevalence of infection is dependent on the body condition of goat. Highest parasitic prevalence was found in the goat with poor body followed by moderate and good body conditions of the host. Similar study also found in many researches like Biswas *et al*., (2014) and Admasu and Nurlign, (2014), who have repoted higher parasitic prevalence and infection rate in hosts with poor body condition more than moderate and good condition. Dabasa *et al*., (2017) have reported that highest parasitic prevalence in good body conditioned host likely than poor and medium body conditioned host. Poor body condition may be due to the poor immunity, malnutrition and poor pasture management response to the productiveness of the parasites (Watson *et al*., 1994; Keyyu *et al*., 2006; Van Wyk *et al*., 2006; Negasi *et al*., 2012 and Gonfa *et al*., 2013). Knox *et al*., (2006) observed that a good body condition was not infected with worms, and usually a poor diet resulted more parasitic infections in host (Islam *et al*., 2017). Many researchers were identified with the present also were represented same study (Temesgen (2008), Keyyu, (2003) and Ahmed (1988). Sissay (2007) who also have reported that higher

prone conditions of parasites increase the rate of mortality of host and loss the production in industries. Kumba, *et al*., 2003 and Bedada (2017) reported that poor management and grazing system lead to a loss of craving, reason of loss of meat industries and goat rearing.

**Table 3.11 Prevalence of parasitic in accordance to body condition of goats**

| **S.no.** | **Body Condition** | **Total no. of examined intestines** | **No. of infected intestines examined** | **Percentage of infection** |
|---|---|---|---|---|
| **1.** | Poor | 540 | 217 | 40.18 |
| **2.** | Moderate | 540 | 152 | 28.14 |
| **3.** | Good | 540 | 171 | 31.66 |
| **$X^2$=18.62 (p< 0.05)** | | | | |

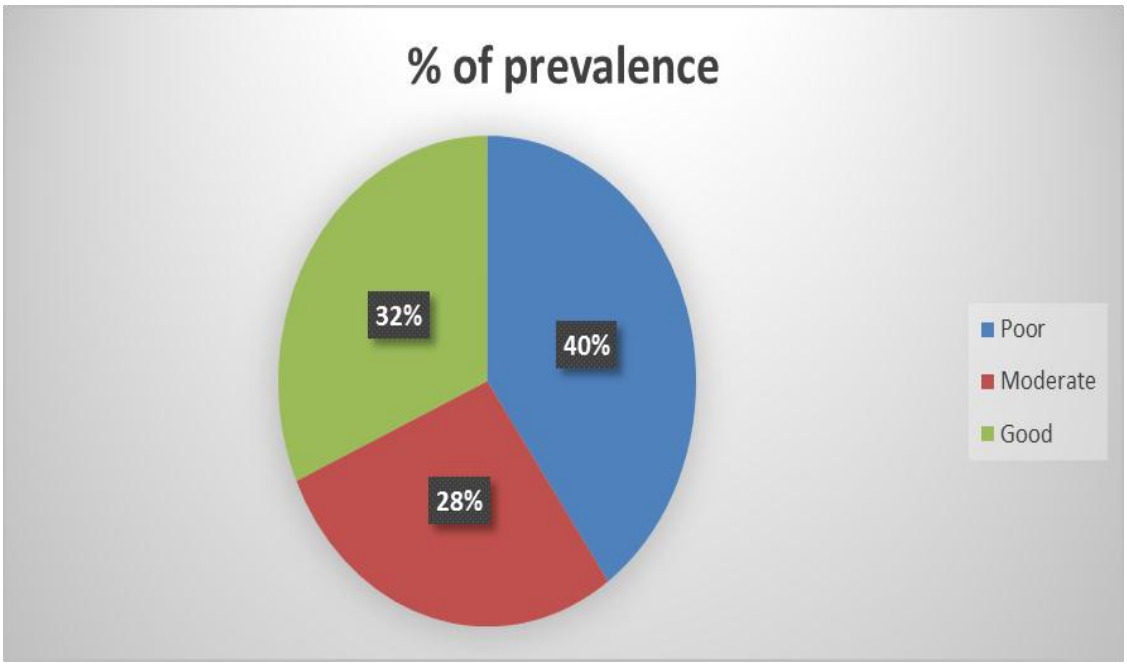



**Figure 3.11 Percentage prevalence of infection in accordance to body conditions of goat**

### 3.3.10 Egg identification of nematode parasites in goat

In this study, the faecal examination showed high frequency of nematode eggs counts in host. Faecal (EPG) examination indicated that the higest parasitic burden in poor condition, 244/540 (45.18%) compared to the moderate 118/540 (21.85%) and good codition 7/540 (1.29%) of gastrointestinal tract. Statistically data was also showed the nonsignificant variations in this study ($X^2$=5.99**,** p>0.05). The prevalence of eggs of parasites was also studied in many researches Ogbe *et al*., (1990), reported that faecal egg count is not reliable suggestion of significantly reporting the parasites burden. The intensity increases of eggs are based on the seasonal variation and conditions of ecological factors (Githigia *et al*., (2001); Okoli *et al*., (2006); Aliu *et al*., (2001) and Abubakar, (2002). The highest parasitic eggs were counted in rainy season and humid conditions (Nhacumbe and Sitoe, 2019). Similar results also were found in many researches (Mbaya and Udendeye, 2011, Helenbrook *et al*., (2015); Kouassi *et al*., (2015); and Hoste *et al*., (2002). Fivaz *et al*., (1990); Agyei (1991) and Nwosu *et al*., (1996) also reported highest parasitic eggs prevalence during the rains. Soulsby (1982); Ross and Smal (1980) and Tripathi (1980) reported that low temperature leading to no development of eggs. Suarez *et al*., 2017 have reported that during lactation period goats have significantly high faecal egg counts (Armour, 1980; Connan, 1976; Rossanigo and Frigerio (2000). Rahman and Collins, (1992) also reported that egg counts of pregnant goat were significantly higher compared to non-pregnant goats.

### 3.12 Egg identification of nematode parasites in goat

| S.no. | Body Condition of goats | Total no. of tested with eggs/ Total samples | Percentage (%) of infection | Samples | Average egg per gm in faeces of goat |
|---|---|---|---|---|---|
| **1.** | Poor | 244/540 | 45.18 | 4g | (av.12 eggs/g) |
| **2.** | Moderate | 118/540 | 21.85 | 4g | (av.10 eggs/g) |
| **3.** | Good | 007/540 | 01.29 | 4g | (av.1.0eggs/g) |
| **$X^2$=5.99 (p>0.05)** | | | | | |

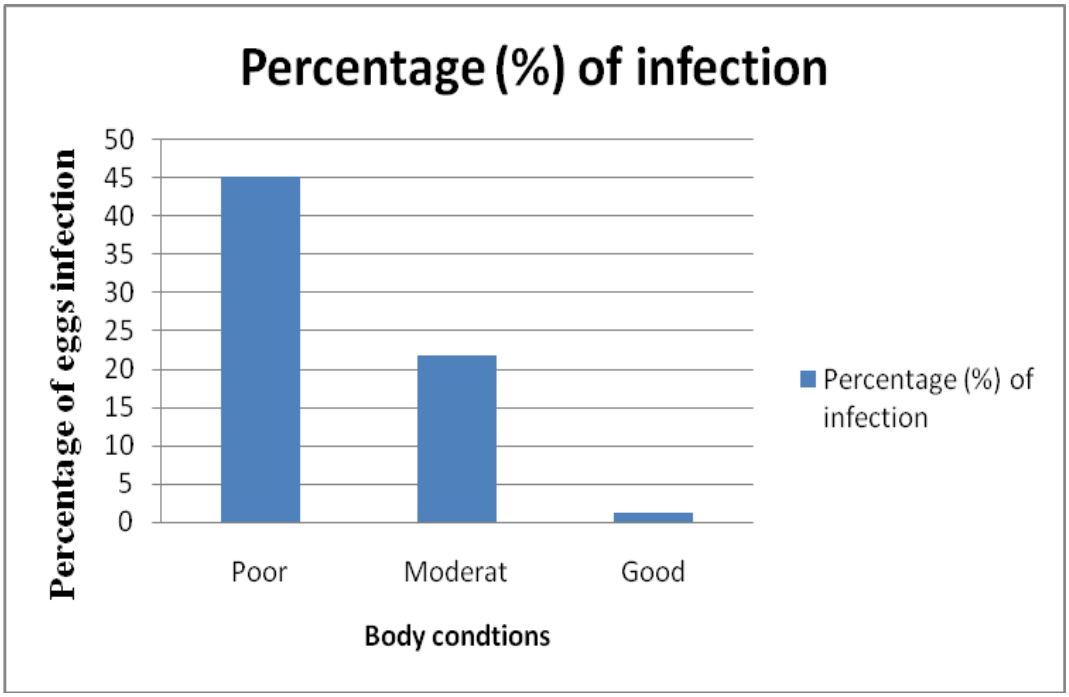



**Figure 3.12 Egg identification of nematode parasites in goat**

### 3.4 Conclusion

Helminthiasis parasitic infection is one of the major constraints in goat industry contributing to maximum economic losses in Lucknow (Uttar Pradesh). The present study an epidemiological study of various helminth parasites was found in different breed of goats. In this study, it was found that gastrointestinal nematode parasites in small ruminants are highly prevalent and which is found to be the crucial cause of economic losses in the goat production. Some control measures for gastrointestinal parasites can be undertaken to reduce the intensity of the infection. In this regard, it is suggested that practice of separate grazing of an animal with low stocking rate and proper deworming at regular intervals may be adopted to overcome the losses and reduce the infestation rate. Furthermore, during the wet season (July, August and September) climatic factors like temperature and humidity are found to be best for the development and survival of parasitic stages of nematodes, for that reason, it is suggested that anthelmintic treatment on quarterly basis (regular deworming) may be implemented to reduce the risk of infection. Necessary steps should be taken to combat parasitic infection in timely manner to improve the productivity from these animals. New technology should be adopted for the diagnosis of parasites.

# Chapter-4

# To study the morphological identification of gastrointestinal nematode parasites of goats

# CHAPTER-4
# TO STUDY THE MORPHOLOGICAL IDENTIFICATION OF GASTROINTESTINAL NEMATODE PARASITES OF GOATS

## Introduction

Gastrointestinal parasitic infection are major problem of the world which is reducing the productivity of the livestock industry in developing countries, including, India. (Gall, 1981; Biu *et al.,* 2009 and Jegede *et al*., 2013). Helminthic parasites infestation is the greatest significant preventive aspect of production in the goat industry (Gadahi *et al.,* 2009) due to the morbidity, cost of treatment and mortality and control measures on a clinical and subclinical level (Nwosu *et al*., 2007). Due to unhygienic condition, improper care and contact with infected animals, ruminants get infected with parasites (Gadahi *et al.,* 2009). Goats are more prone to other pathogenic infections leading to heavy economic losses (Garedaghi *et al.*, 2011).

Nematode parasites are mentioned to be the common and most important gastrointestinal parasites in livestock (Maingi, 1995). Nematodes have slightly flattened cylindrically body; hence it is called roundworm. Nematodes are usually bisexual. These parasites are most prevalent parasites in this study area, leading to loss of productivity and poor growth (Pedreira *et al*., 2006). Nematodes are also widely distributed in other countries like Asia, Africa and some Mediterranean countries (Sharkhuu, 2001), Kazakhstan (Morgan *et al*., 2006), Saudi Arabia (El-Azazy, 1990 and Magzoub, 2000), Namibia (Krecek *et al*., 1990), Turkey (Yukari, 2005) and Iran (E slami *et al*., 1976). The prevalence of gastro-intestinal nematode parasitic infection based on agroclimatic conditions like temperature, quality and quantity of pasture humidity, and pasture management of the animals (Pal and Qayyum, 1993). Gastrointestinal nematode parasites are epidemics associated with the goat infection during monsoon season being more infected than the winter seasons. These parasites may be overwhelming at subclinical level (Barger *et al*., 1994).

The parasitic infections enhanced susceptibility encompasses mortality, morbidity and losses in goat industries (Herlich, 1978). It increases economic status of the rural poor. As gastrointestinal parasite infection is the most important limiting factor of sheep productivity, parasitism has a highly detrimental effect on the sheep industry (Jones, 2001). Economically, loss of productivity up to 15% and weight loss of the particular infected host up to 50% caused by gastrointestinal parasitic infection have been reported by Hussain, 1985; Bhat *et al*., 2011 and Shahnawaz *et al*., 2011.

Diagnosis of parasitic nematode infections of ruminants, both qualitative and quantitative, is dependent on inaccurate methods such as faecal worm and egg counts and identification of the parasites (Van Wyk *et al.*, 2013).

The study revealed that the infections were cuased by *Haemonchus sp.*, *Oesphagostomum sp.,* and *Trichuries sp.* etc. and mixed infections were the most prevalent. The definitive classification is based on the external and internal morphology of egg, larval and adult stages (Gilbert, 1996). Morphological identification of parasites was reported by Soulsby (1965). Observations of the parasites were total body length, spicules, cervical papillae, vulval flap and cervical lamina were made on worms prepared on permanent mount slides (Patchamuthu, 1993).

Identification of individual worms was done with the help of Microscope and Scanning Electron Microscope (Rahman and Hamid 2007). Ultrastruture of nematode parasites and their eggs were determined using Compound microscope and SEM, its classification was done based on some species specific characters, shape and size of parasites and their eggs.

## 4.2 Materials and methods

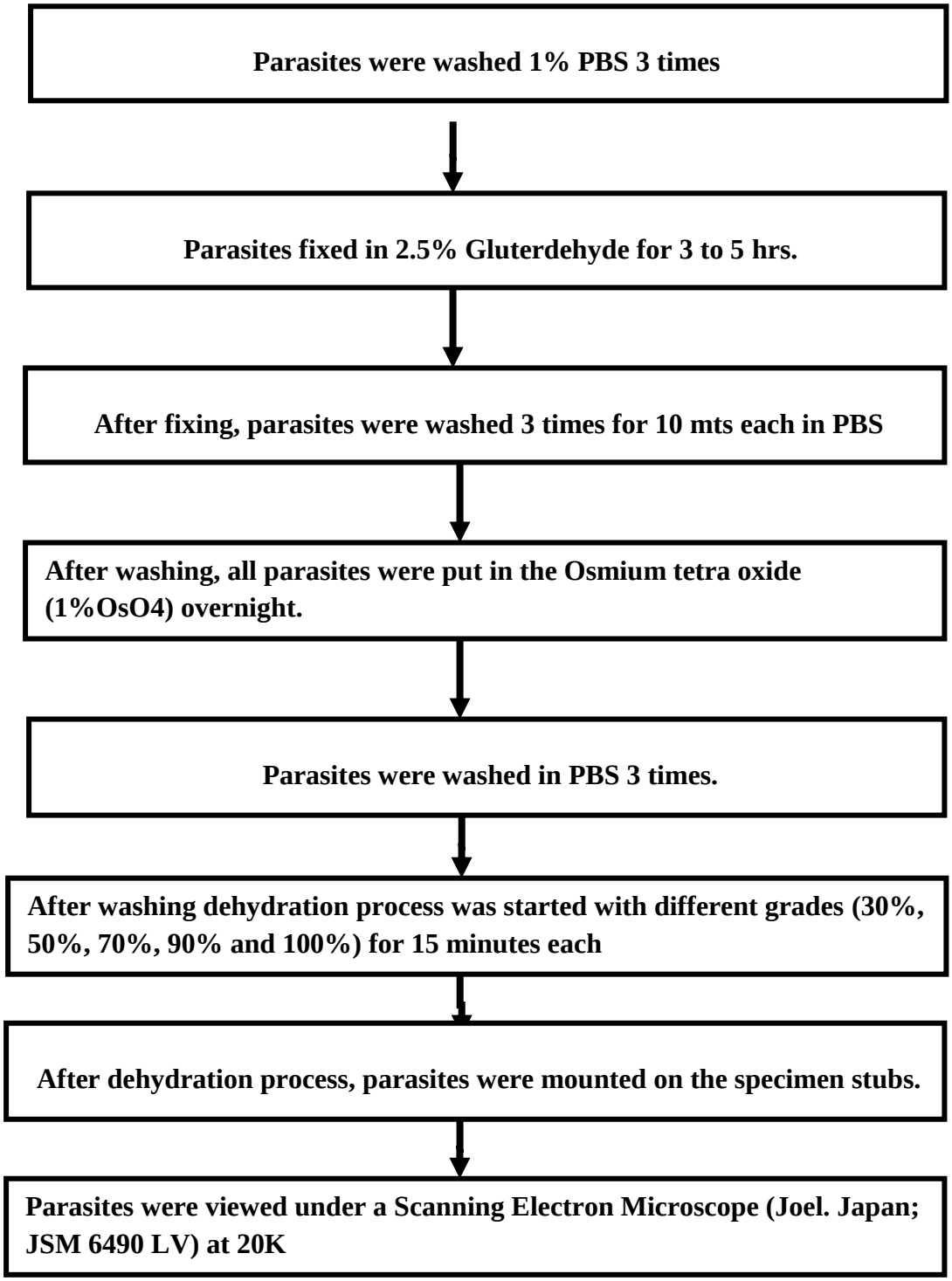
### 4.2.1 Mounting of parasites

Parasites were mounted in glycerol and images were taken under the light microscope (10x and 40x) and identified with help of technique given by Soulsby (1982).

### 4.2.2 Scanning Electron Microscopy (SEM)

Following the standardized Scanning Electron Microscopic (SEM) protocols the experiments were performed at the University Science Instrument Centre (USIC), Babasaheb Bhimrao Ambedkar University, Lucknow (Uttar Pradesh).

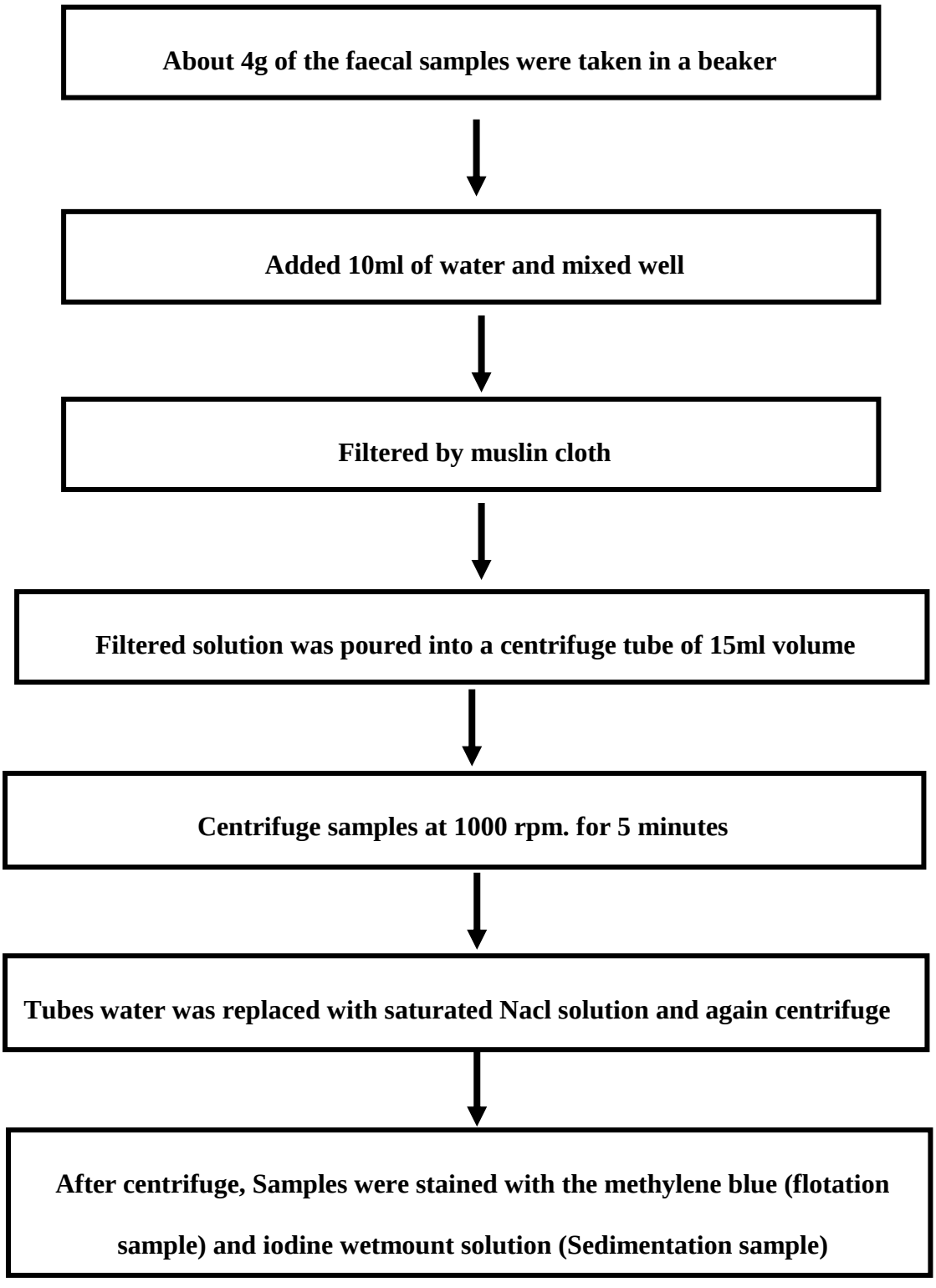
The following Protocol was followed by standard protocol, developed by Dey *et al*., (1989) and Roy and Tandon (1991).



### 4.2.3 Examination of eggs

Faecal sample was collected from gastrointestinal tract of goat. Collected sample was examined for presence of egg with the help of sedimentation and flotation techniques with standard method proposed by Soulsby (1982).

**Sedimentation and floatation**



**Then take drop of sample kept on the slide and cover slip placed and examined and confirmed the eggs in faecal mater**

**Prepared slides were observed under microscope (10X and 40X)**

**Eggs of different nematodes were identified on the basis of morphological appearance (eggs colour, shape and size.)**


**Figure 4.1 (A) Centrifuge unit (B) Centrifuge tube filled with fecal matter of goat**

## 4.3 RESULTS

In this study, it was revealed that gastrointestinal nematode parasites and their eggs, were on identified morphologically (microscopic and SEM). However, current results indicated that nematode parasites are most prevalent parasites of which and only 3 genra were found in this study area, including *Haemonchus* spp., *Trichuris* spp. and *Oesophagostomum* spp.

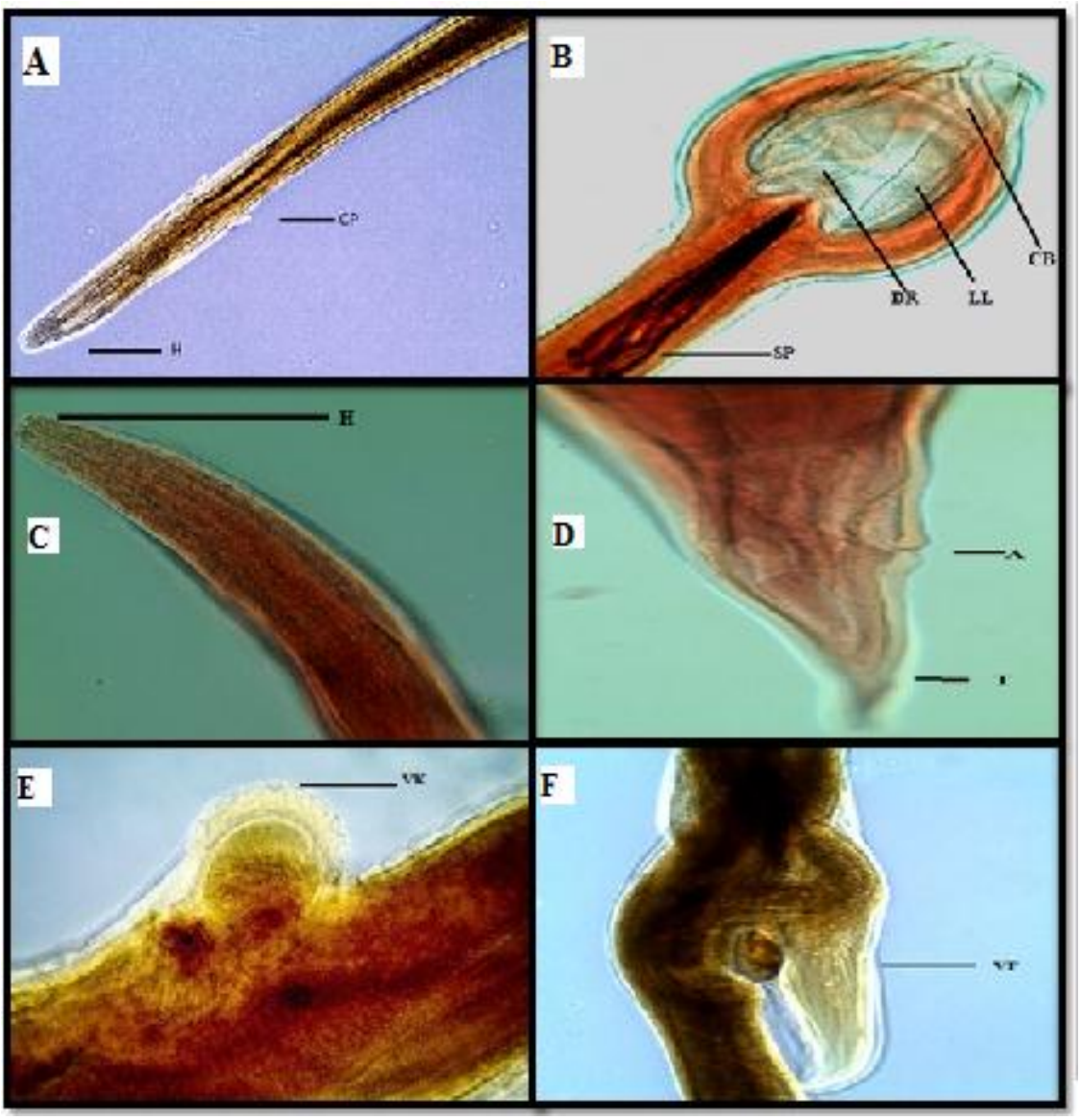



**Figure 4.2 (A) Anterior end of *Haemonchus* spp., ventral view, showing prominent paired lateral cervical papillae (CP) (B) Posterior region of male Dorsal ray (DR). (C) Anterior region of male (D) Female posteror region showing anus (A) and tail (T). (E) Lateral view showing female vulvar nob (VN) (F) Vulvar flap (VF).**

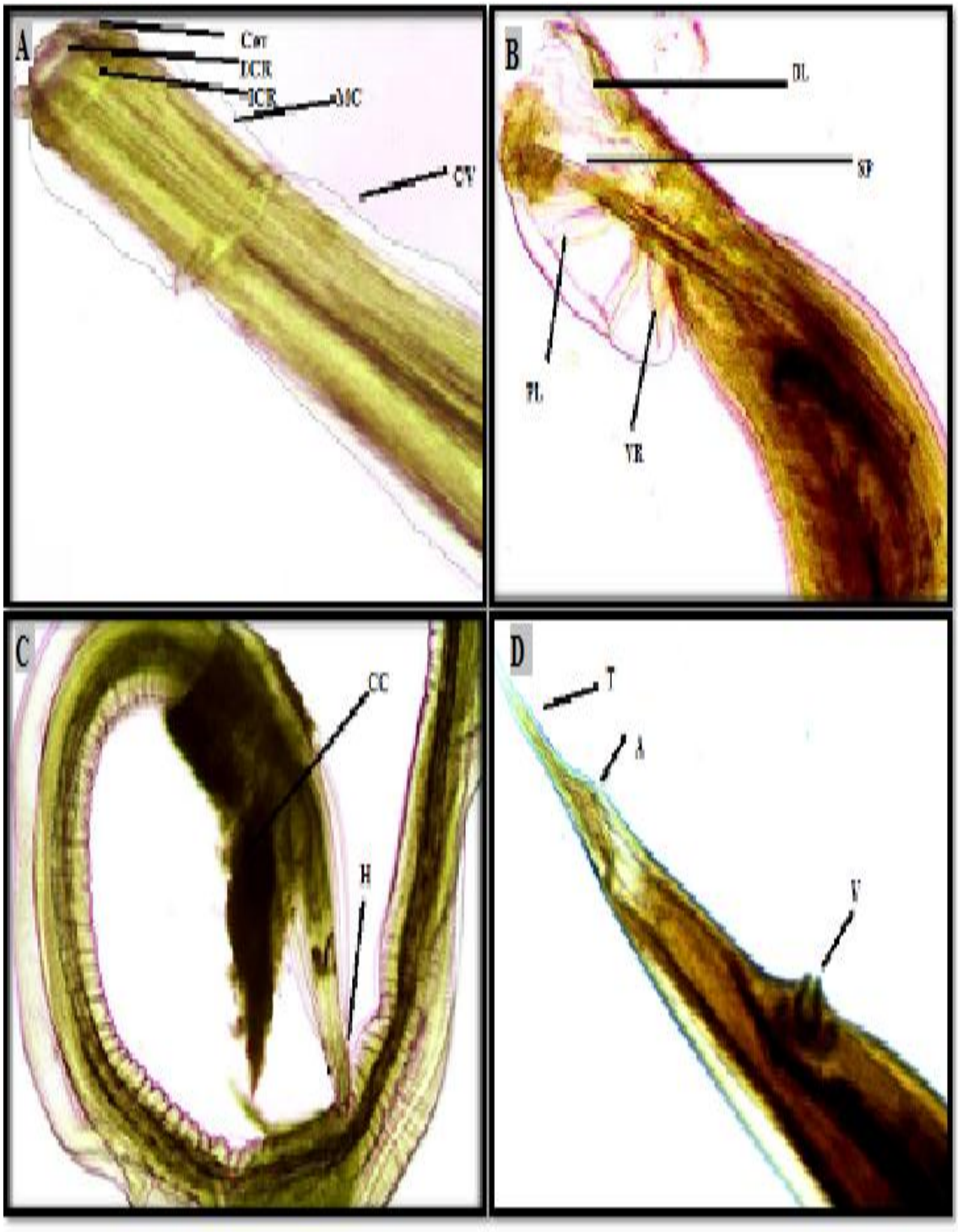



**Figure 4.3 (A) Anterior end of *Oesophagustomum* spp. Cephalic vesicle (CeV), Internal Corona Radiata (ICR), External Corona Radiata (ECR), (B) Posterior part of male Dorsal lob (DL), (C) Anterior part of female- Capulatory Cement (CC) (D) Posterior part of female Vulva(V).**

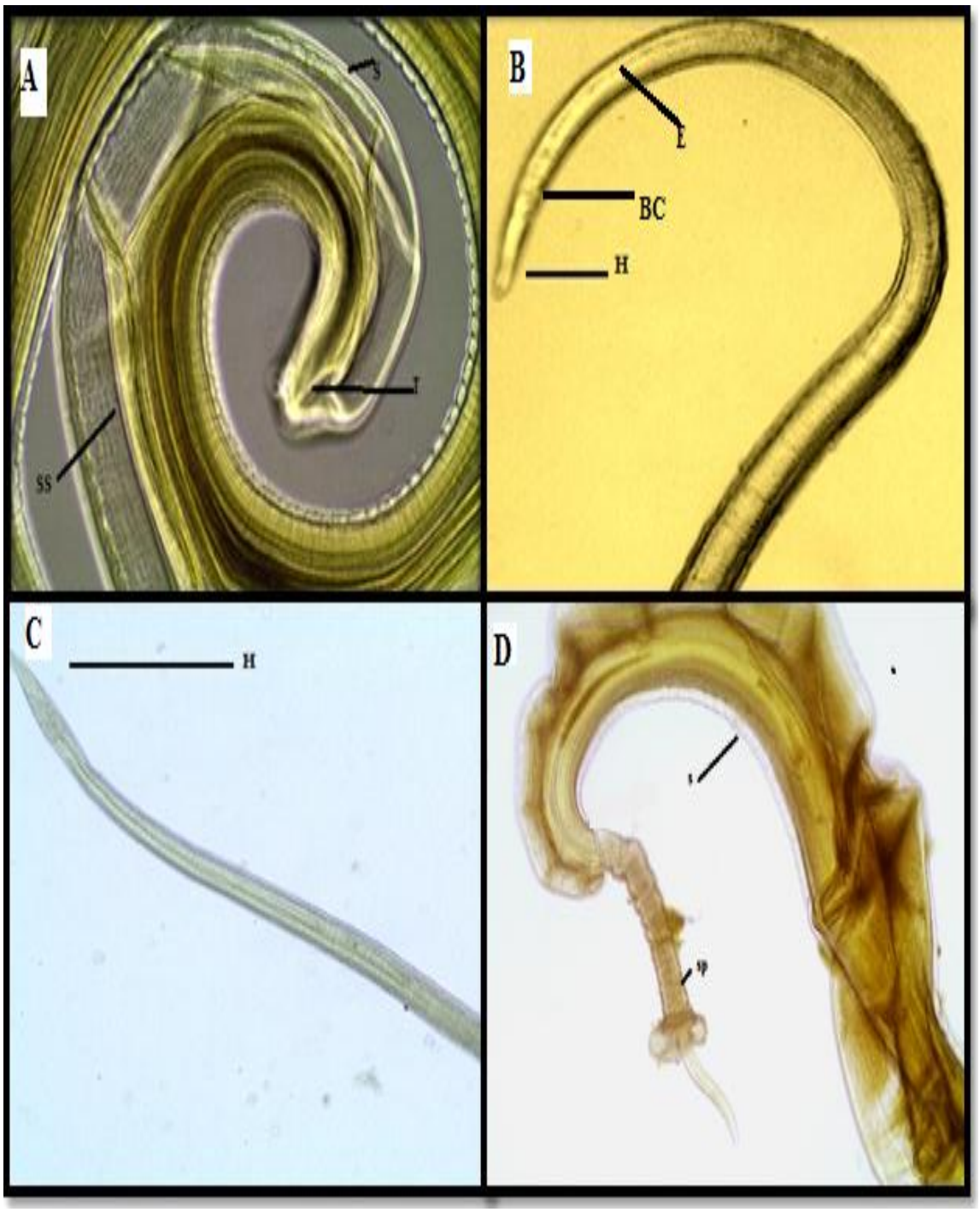



**Figure 4.4 (A) Anterior region of male showing Spicule sheath (SS), Spin (S) (B) Anterior region of female showing Buccal Capsule (BC) and Esophagus (E) (C) Anterior region of head of male (D) Posterior region of male showing spicules(S) and capulatory bursa (CB).**

**Eggs of parasites**

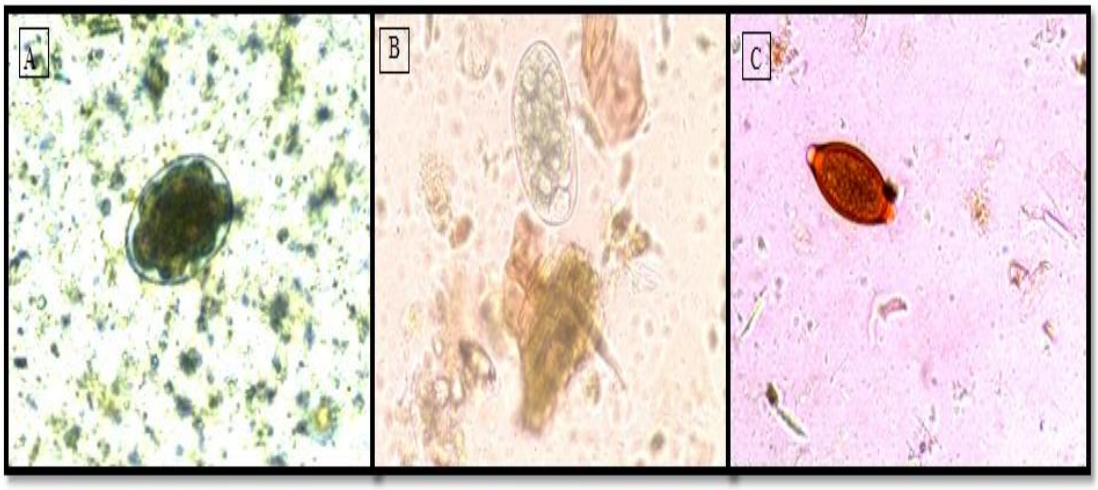



**Figure 4.5 (A) Egg of *Haemonchus* spp. (B) Egg of *Oesophagostomum* spp. (C) Egg of *Trichuris* spp.**

### 4.5.5 Ultra-structure study of nemtode parasites

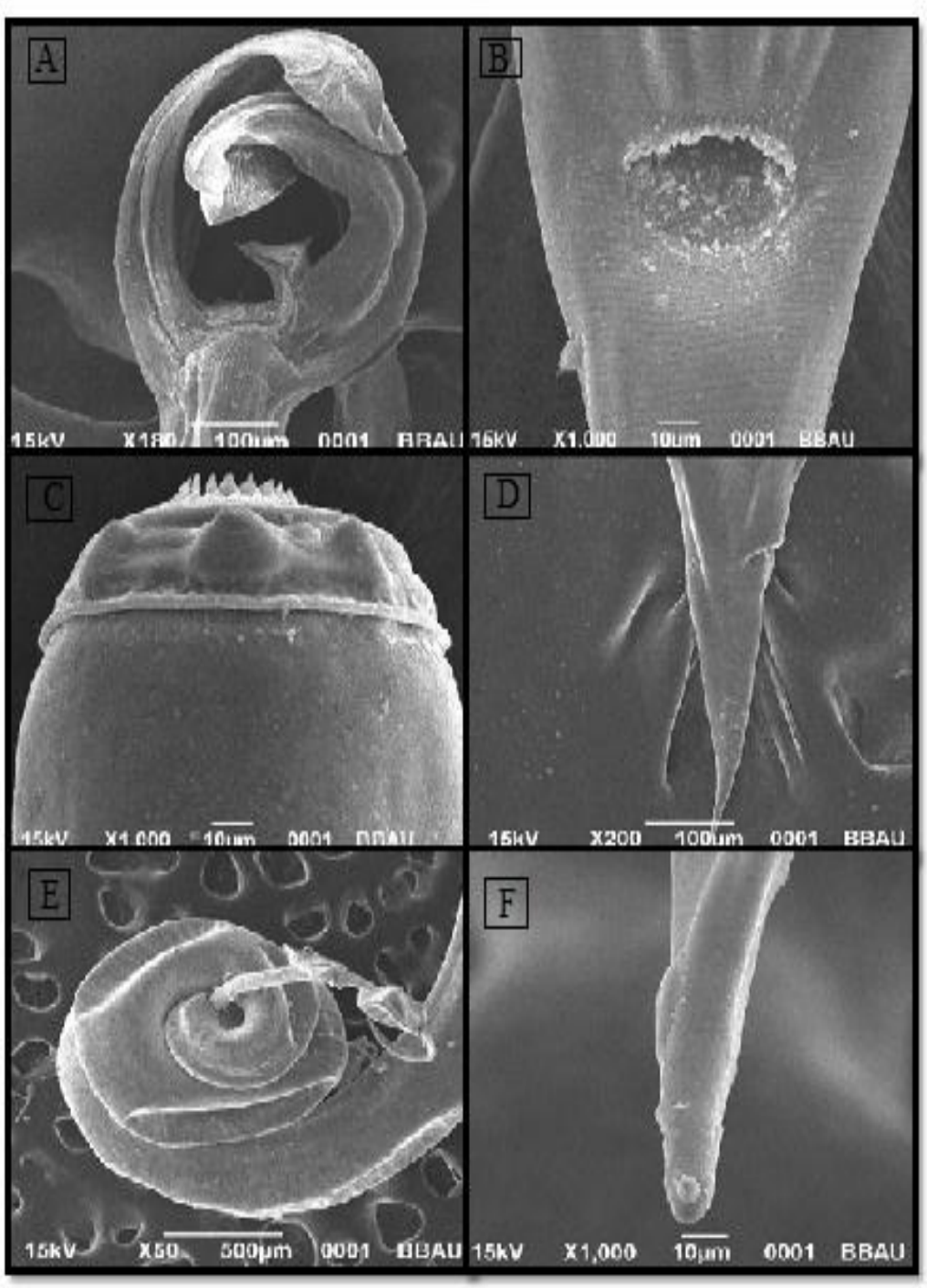



**Figure 4.6 SEM (Scanning Electron Microscope) Micrograph showing- (A) Capulatory bursa of *Haemonchus* spp. (B) Cephalic papilae (CP) and mouth part of *Haemonchus* spp. (C) Amphid (A) and Depressions (D), leafs of external corona radiate (ECR), leafs of internal corona radiate (ICR), mouth capsule (MC), mouth collar (MC) of *Oesophagostomum* spp. (D) vulva (V) of female and sharp pointed tail (T) of *Oesophagostomum* spp. (E) Posterior region showing (SP) of male of *Trichuris* spp. (F) Haed (H) of *Trichuris* spp.**

### 4.3.1 *Haemonchus* spp.

| |
|---|
| Kingdom : Animaliya |
| Phylum: Nematode |
| Class:Secernentea |
| Subclass: Rhabdita |
| Order: Strongylida |
| Family : Trichostongy |
| Genus: *Haemonchus* |

***Source:*** *wikipedia and Rudolphi, 1803 and Cobb, 1898.*

*Haemonchus* spp. is a very common nematode parasite, found in the Abomasums of the goat. It is also known as the barber's pole worm and blood sucking parasite and its infection, called haemonchosis. Adult larvae get attached to the abomasal mucosa in the host. This parasite is responsible for oedema, anemia and death of infected host. It is mainly prevalent in hot, humid climates condition.

- Body shape is cylindrical and the female parasite is longer (18 to 30 mm) than the male (10 to 20 mm).
- Males have an even, reddish color and a bursa with an asymmetrical dorsal lobe, barbed spicules (460-506 µm long) and Y-shape dorsal ray.
- It has prominent cervical papillae. Cuticle is transverely striated. Longitudinal indistinct striations are also present. They have small buccal cavity with small dorsal lancet or teeth.
- Female have vulvar flap is usually enclosed by a linguiform form (vulva flap) which is very prominent and large.

### 4. 3.2 *Oesophagostomum* spp.

| |
|---|
| Kingdom : Animaliya |
| Phylum: Nematode |
| Order: Strongylida |
| Family : Strongyloidae |
| Genus: *Oesophagostomum* |

***Source:*** *wikipedia.*

*Oesophagostomum* spp. are most prevalent in the worldwide. *Oesophagostomum*, are found in the large intestine of goats and others animals. The disease they cause, name is oesophagostomiasis, is known for the nodule creation in the intestine.

- Adults worms are normally stout and white,
- Female parasites being larger than the male.

Size - Male 1.5-03 cm

Female 02-04 cm

- *Oesophagostomum* spp. contains a developed, multi-nucleate digestive tract as well as a reproductive system.
- Males can be distinguished by their bell-like copulatory bursa, located in the tail, and their paired rod like spicules.
- The anterior end of the parasites contains a striated cuticle occurred between the mouth and excretory pore.
- The mouth is bounded by oral papillae and cylindrical buccal capsule, as depicted above, is surrounded by a protective "external leaf crown" that is called the corona radiata.

### 4.3.3 *Trichuris* spp.

| |
|---|
| Kingdome : Animaliya |
| Phylum: Nematode |
| Class: Enoplea |
| Order: Trichocephalida |
| Family: Trichuridae |
| Genus:Trichuris |

***Source:*** *wikipedia and Roederer (1761)*

*Trichuris* spp., has a narrow anterior and pinkish-white worms are threaded through the mucosa. These parasites also feed on blood and are found in large intestine.

- Esophageal end and shorter and thicker posterior end.
- Males are shorter than females.

Size- Male 3-4 cm

Female 4-7 cm

- In male worm, the posterior end has spirally coiled with a single spicule, proximal and a pointed distal end. Spicular sheath (4.8-6.0 mm long) had a slightly stretched globular expansion at the end of distal and it is also covered with the spines.
- Female caudal extremity is either shaped slightly curved liked "comma" and arc. but vulvar opening of female worm in oesophageo intestinal junction with genital tract are bearing fully developed eggs in a single profile.
- Oesophagus consists of a thin walled tube surrounded by large unicellular glands, called the stichocytes
- Buccal cavity having spear like structure which are devoid of lips. The cuticle was transversally striated with longitudinal bacillary band.

### 4.4.4 Egg Identification

Eggs of different Nematodes (*Haemonchus* spp., *Oesophagostomu* spp., *and Trichuris* spp.) were identified on the basis of morphological appearance shape, color and size.

Egg of *Haemonchus* spp.- The adult female parasite can release between 5,000 and 10,000 eggs, which are passed out from the feces. Eggs are growing in humid conditions in the gastrointestinal tract of goat. Eggs are yellowish in color and oval in shape with both ends having equal poles and morula not fully filled with the cavity of the egg. Average length of eggs are 74 to 95 µm long and 35 to 60 µm wide, and the early stages of eggs (cleavage) contain between 16 and 32 cells (Figure 4.5).

Eggs of *Oesophagostomum* spp. are indistinguishable from hookworm eggs. They have a thin shell and with size ranging from 50 to 75 µm long and 35-45 µm wide.

Eggs of Trichuris spp. have both polar plug look like a lemon shaped. Their characteristic eggs are barrel in shaped and brown, and have bipolar protuberances.

The Nematode eggs consists of 4 layers on the shell, an outer uterine layer, a vitelline layer, a chitinous layer and an inner lipid layer (Floor, 1967) but all shells are not occurred in all genra of eggs, like *Haemonchus* spp., *Oesophagostomum* spp. and *Trichuris* spp. chitinous layer is thick layer that composed of a chitin complex that provides structural potency (Gaugler and Bilgrami, 2004).

**4.4 Discussion**

Nematode parasites are typically elongate, tapered at both the ends, dorsoventrallly and bilaterally symmetrical. The parasites would greatly improve the diagnosis and controlling the pathogenic world wide. Total body lengths of both sexes, cervical papillae of females and both spicules of males parasites (Fig 4.2) were significantly longer. Monnig (1931) studied and made a comparison between different stages of parasites of the various genera and species that will enable rapid identification with the minimum number of measurements. It is also necessary to explain characteristic feature of focus by observation under microscope (Light microscope and Scanning Electron Microscope (SEM).

Cobb 1898, was the first one to discriminate *Haemonchus* spp. (Rudolphi, 1802). Similar studied was found in many researches given by Sahai and Deo (1964) and Soulsby (1986). (Zahida 1992, Reyaz, 2005 and Kuchai, 2012 and Degheidy 2014, suggested that morphological and morphometric characters including total length, colour, maximum width, female genital apparatus, shape of bursa lobes shape of spicules. *Haemonchus* spp. was recognized as the dominant and most prone species in goats. This result was signified by Achi *et al.,* (2003). *Haemonchus* spp. are most effective and prevalent parasites in this area. Comparatively study hosts knobbed and linguiform were well balanced with a slight preponderance of knobbed females. Similar findings have been reported by VanWyk *et al*., (2004). Lichtenfels *et al*., (1994) and Jacquiet *et al*., (1997) also discussed morphological identification of males and females parasitic species. The characteristic features in male and females included size, body length, oesophagus length, spicule, left and right spicule barb length distance of cervical papillae from head, gubernaculums, oesophagus

length as a percentage of total body length, vulval morphology tail length, and the distance of the right and left phasmid from the distal tip of the tail (Vadlejc, 2014). Lichtenfels and Pilit (2000) reported that external surface of nematodes species were determined by characters of *Haemonchus* spp. and relationship of different species. The linguiform and value for distinguishing in *Haemonchus* spp. from sheep (64%) and knobbed from goats (50%) (Lichtenfels *et al*., 1986). Tod, 1965 reported that the difference in vulvar structure of female *Haemonchus* spp. worms were examined from the gastrointestinal tract of host (sheep and goats) and identified by the genetic factors. Similar findings were reported also by Jacquiet (1998), Kuchai *et al*., 2012 and 1998, Bersissa (2004), Rahman and Hamid (2007), Kumsa (2008) and Gharamah et al. (2012) and Akkari *et al*., (2013). The linguiform, sublinguiform and vulvar flap structure of *Haemonchus* worms collected from goats are most prominent type. Identification of male and female *Haemonchus* spp., Das and Whitlock (1960) described the diverse types of the vulval (linguiform with, 2-knobbed, knob-like, and 3-smooth form, without a vulval). Gharamah *et al*., (2011) reported the cervical papillae, spicule and valvar flap of *Haemonchus* spp. Akkari *et al*., (2013) also studied the vulvar flap of female and spicules of male of *Haemonchus* spp. worms of goats.

*Oesophagostomum* parasites were most prevalent in this area and these parasites are found in large intestine of the host. Morphological identification of parasites showed that collar is present in center of the mouth, which is bounded by an outside leaf crown structure, from 14 to 16 element and 28 to 32 elements inside leaf crown, whereas it appears to show lobed or bilobed region, and a trilobed also found to be placed in head region. The number of buccal leaves of the corona radiata at the anterior mouth opening of *Oesophagostomum* spp. are difficult to observe with the light microscope but can be easily seen by Scanning Electron Microscope (SEM). Yadav, (2006) also showed the similar study that cephalic papilla, external corona radiata, lateral amphid, bursa, genital cone, oral collar, external dorsal and anterior lateral rays, anus, vulva, and caudal papillae match structurally up with our findings. The similar study is reported by Goodey, 1924; Daubney, 1924 and Neuhaus *et al*., 1997. Gamit, 2018 also reported the similar findings that worm body is white coloured and 8.00 mm length size and are found in large intestine of host, however microscopic assessment showed the cephalic vesicles and cervical alae

notch at one side. Head collars region were found in the anterior end of the parasites (Fig. 4.6). Eggs were examined from the present in faecal sample collected from intestine.

Genus *Trichuris* is a nematode parasite. These parasites spreading many infectious diseases and depend on different climatic and geographic factors (Soulsby 1982; Anderson, 2000; Bethony *et al*., 2006 and Ghai *et al*., 2014). The morphological studies also revealed observations of trichuries like spicule, thinner distal end and rounded. The Male *Trichuris* spp. have proximal spicule sheath and vulvar flap in females. Papillary processes were characteristic near the vulval opening. Morphological identities of parasites also are represented by Afrin *et al.*, (2016). Infection of *Trichuris sp.* in large intestine of the host caused the thickening of mucosa which was experiential during autopsy. Mohanta *et al*., 2007 also reported that the gastrointestinal parasitic infection showed haemorrhagic spots, congestion, and ulcer formation. Mucosa of the caecum has severe inflammation and sometimes colon was experiential in the host caused by parasites (Soulsby, 1982; Bowman 2002 and Taylor *et al*., 2007). Kumar, 1987; Lal, 1987; Saha, 1998; Bhowmik, 1998; Patnode *et al*., 2014 and Kumar *et al*., 2015) also reported similar findings occasional peripheral eosinophilia, tissue invasion, hyperemia, hemorrhages and lymphocytic infiltrations in the mucosa. Taylor *et al*., 2007 and Yevstafieva *et al*., 2019 have reported that species identification of parasites based on genomic specific character of parasites based on morphologicaly. Morphometric parameters are used for the identification of the shapeand size of the male and female species (Zainab and Khan, 2016).

Identification of nematode parasites eggs based on shape, size and colour was using the microscopic study. Adaptations and infection rate of nematode parasites and eggs in the gastrointestinal tract of the goat based on the environmental factors was also studied (Wharton, 1980 and Qazi *et. al.,* 2020). Maurelli *et al.*, 2006, reported 100% detection of the common gastrointestinal nematodes in animals (*Haaemonchus* spp., *Oesophagostomum* spp. and *Trichuris* spp.). The present study was majorly based on sedimentation and flotatation technique for the eggs collection and identification (Alvarado-Villalobos *et al*., 2017). Bennett (1990) has reported that eggs and parasites show significant relationship between nematode parasites

(Indre *et al*., 2010). Sacher (1966) and Crofton and Whitlock (1965) also reported that the studies on the egg of *Haemonchus* sp. was more infected in humid and wet temperature which were formed to be have high relevence than the other eggs of parasites. Levine, (1963) reported that single egg must spend its entire growth and developmental time on the outer side. Lejambre, (1970); Beer, (1973); Jurasek *et al*., 2010 and Sapp *et al*., 2018 have also reported similar findings. The transmission of nematode parasites eggs from faeces to the host often increases the infection and mortality rate of the host (Wharton 1980). Gaugler & Bilgrami (2004) have reported that the nematode eggs have thick layers for protection (Floor, 1967).

## 4.5 Conclusion

The present study encapsulated the morphological identification of gastrointestinal nematode parasites and their eggs. This study was done using Light microscope and ultra-structure SEM (Scanning Electron Microscope). Different specific characters of parasites were described by the light microscope but since all the characters can't be clearly identified by light microscope and Scanning Electron Microscope technique were used to identify Dorsal ray, mouth part, Cephalic papillae, vulva from anterior end and colour and size of eggs. Further study using Transmission Electron Microscope (TEM) and histological parameters were conducted intended for determining their functions.

# CHAPTER-5

# To determine the molecular characterization of gastrointestinal nematode parasites of goats

# CHAPTER-5
# TO DETERMINE THE MOLECULAR CHARACTERIZATION OF GASTROINTESTINAL NEMATODE PARASITES OF GOATS

## 5.1 INTRODUCTION

Nematodes (Roundworms) are also known as strongylids parasites belonging to the order Strongylida, superfamily Trichostrongyloidea. It is found in the gastrointestinal tract in the ruminant animals. This is one of the harmful parasites which is spreading infection to all ruminant animal and causing clinical signs such as anorexia, bloody diarrhea (Wilmsen *et al*., 2014) weight loss, emaciation, hypoproteinaemia, poor body condition, malnutrition and death in the case of heavy parasitic burden (Hale, 2006, Taylor *et al.,* 2007 and Holmes, 1985). Parasitic infestations contribute towards major loss of production and reduced income of farmers in the world (Saraljungstrom, 2017, Hoste *et al*., 1995). Therefore, effective prevention and control of gastrointestinal parasitic diseases are important for the host.

The identification of parasitic species is based on morphological and molecular characterization method. Morphology identification used for some specific characters using molecular methods like genomic, species specific study. Bioinformatics method was a unique method used for better understanding of relationship between two species and host & parasitic interaction (Ahmad, 2011).

Molecular charctarisation is used as a diagnostic tool since 1990s for the identification, quantification and discrimination of the nematode parasites. Data about genetic variation in genomic and species is determined through molecular method (Karp *et al*., 1996). These methods of nematode identification also provide accurate and alternative diagnostic approaches (Punja *et al*., 2008). Based on this method genomic, character of genomic DNA of different species of parasites, PCR and sequencing are identified (Gasser *et al.,* 1993). These techniques are extremely sensitive for accurate identification for parasites up to genomic and species level (Tan *et. al.,* 2014). Molecular techniques have been played a major role in biological studies leading to the understanding of the genetic variation in the

parasites in the host. Logically, it has been also very useful diagnosis tool for the identification of gastrointestinal nematode parasites. The molecular tools such as PCR (Polymerase Chain Reaction) and DNA sequencing are advanced techniques, most sensitive and highly accurate for the identification of nematodes at the genomic level. These techniques are used for detecting specific based on methods have also been genotype organisms. The detection sensitivity of PCR is higher than that of light microscopy; therefore, this technique is useful for detecting a low number of parasites in stool samples. The PCR technique has also been used to investigate non-intestinal parasites (Piarroux *et al.,* 1994 and Duraisingh *et al.,* 1998). Gasser and Newton (2000) reported that genetic variation of the ITS2 (Internal Transcribed Spacer 2) sequences in relatively many parasites. The ITS2 (Internal Transcribed Spacer2) sequences can be used as a tool for species differentiation such as in gastrointestinal nematodes (Stevenson *et al.,* 1995).

The Internal Transcribed Spacer (ITS) region has been confirmed to be very functional for DNA from which universal species-specific primers are used in PCR reactions (Powers *et al*., 1997) and also have very reliable genetic marker for nematode parasitic identification (Gasser *et al*., 1993 and Gharamah *et al*., 2012). Sequence data of the ITS2 is extensively used for the high-resolution marker for several parasitic nematodes (Heise *et al*., 1999; Dallas *et al*., 2000 and Abramatov *et al.,* 2013). The nematode parasitic species can be genetically characterized by rDNA sequences (Chilton *et al.,* 1995). The desirable gene have been encoding with rRNA and conserved with small subunit gene (Highly variable the intergenic spacer regions) (Cutilla *et al.,* 1999 and Dorri *et al*., 1999).

Sequences of the ITS2 (Internal Transcribed Spacer 2) of rDNA have been widely used as genetic markers for the differentiation of many nematodes and identification (Gasser and Newton, 2000; Mejía-Madrid and Aguirre-Macedo, 2011; Lin *et al*., 2012 and Liu *et al*., 2014). The sequence variation in rDNA within a species is significantly less than the levels of sequence differences among species. That trait allows the specific identification of nematodes, including species of *Haemonchus, Trichuris, Cooperia, Nematodirus, Bunostomum, Oesophagostomum* and *Chabertia,* (reviewed by Gasser, 2006 and Gasser *et al*., 2008).

Sequence of parasites is analysed and defined homology by the phylogenetic tree, which is shown in the relationship between different species of parasites taxa (sequences) (Nei and Kumar, 2000; Felsenstein 2004 and Hall, 2011). The method is described by some steps to build up the phylogeny tree from the molecular data. NCBI, BLAST, CLASTAL-W and MEGA-6 version software are used for identifying the molecular evolutionary genetic analysis (Tamura *et al*., 2011).

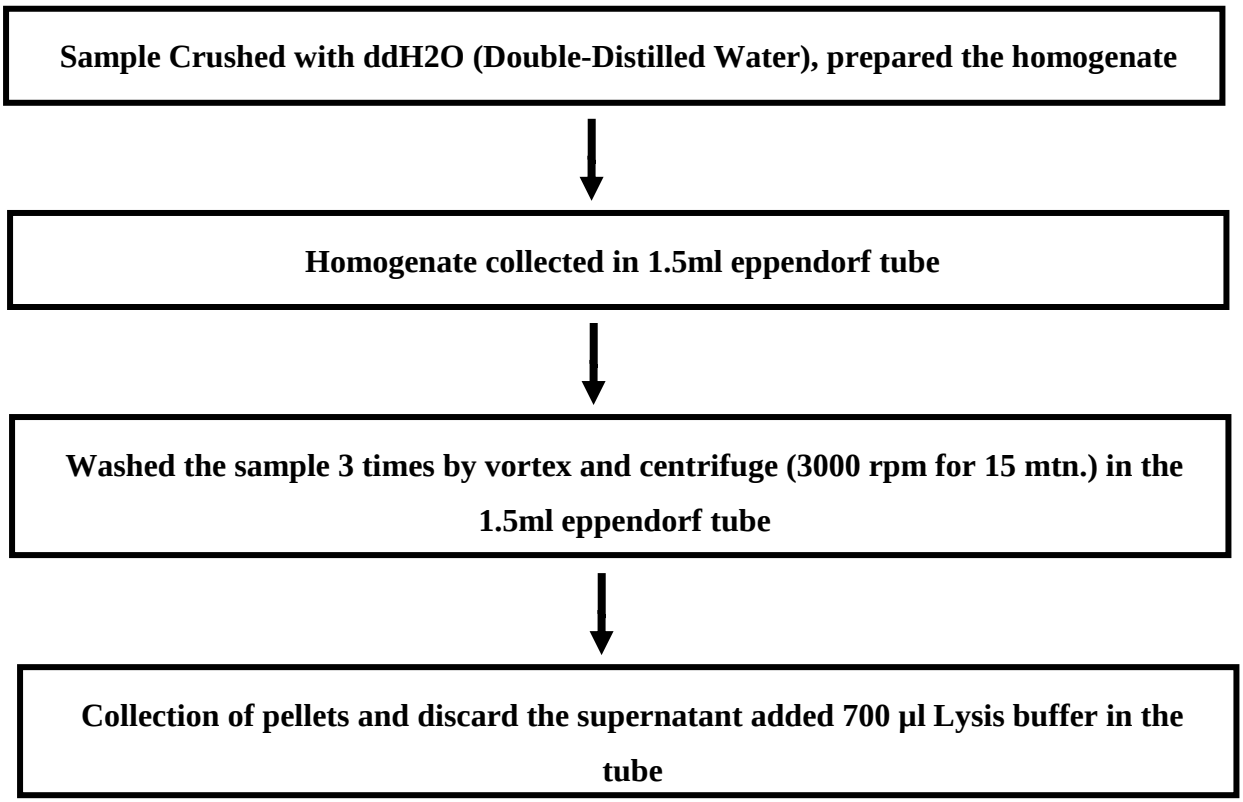
## 5.2 MATERIALS AND METHODS

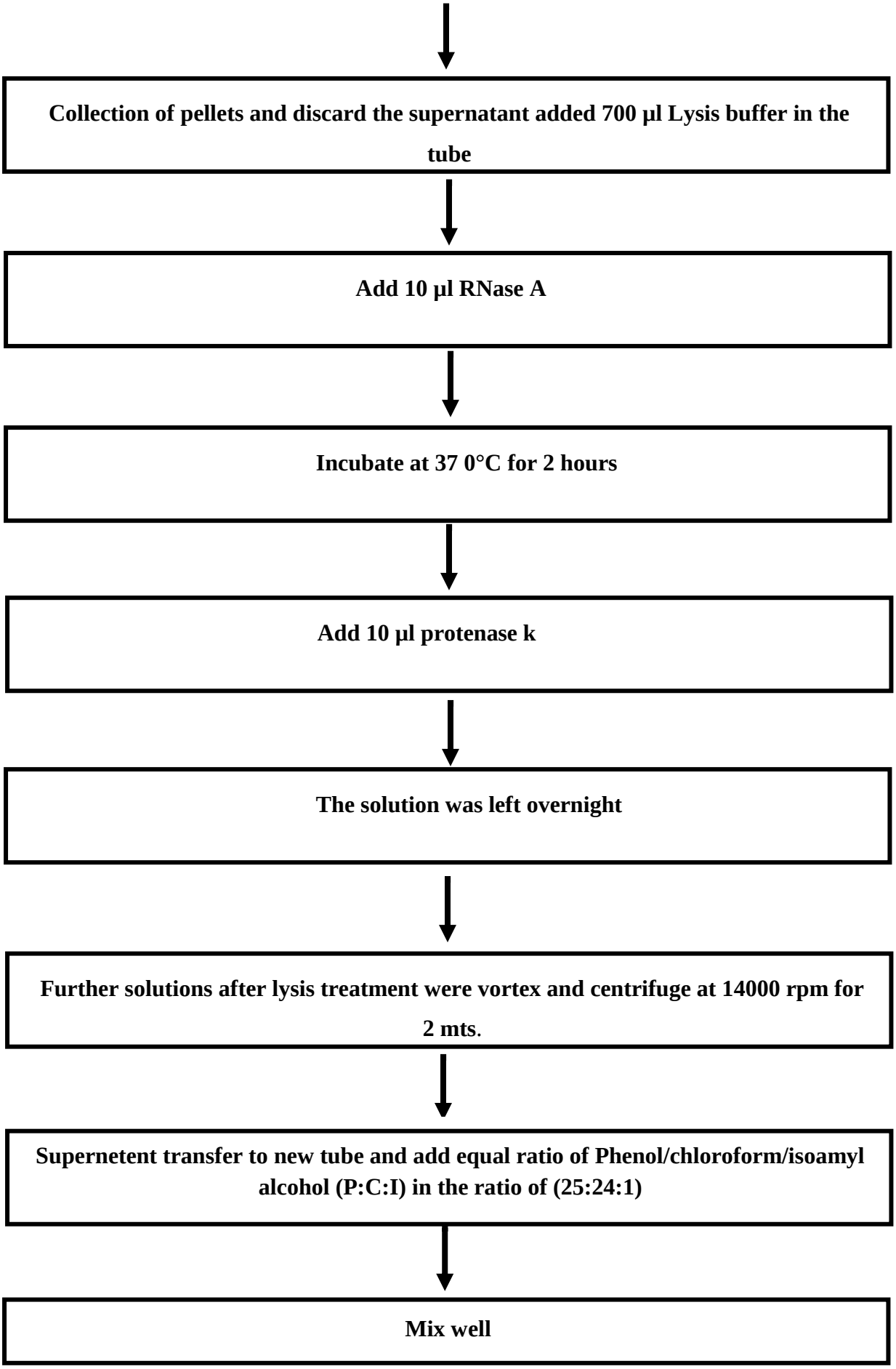
### 5.2.1 Collection and preservation of parasites

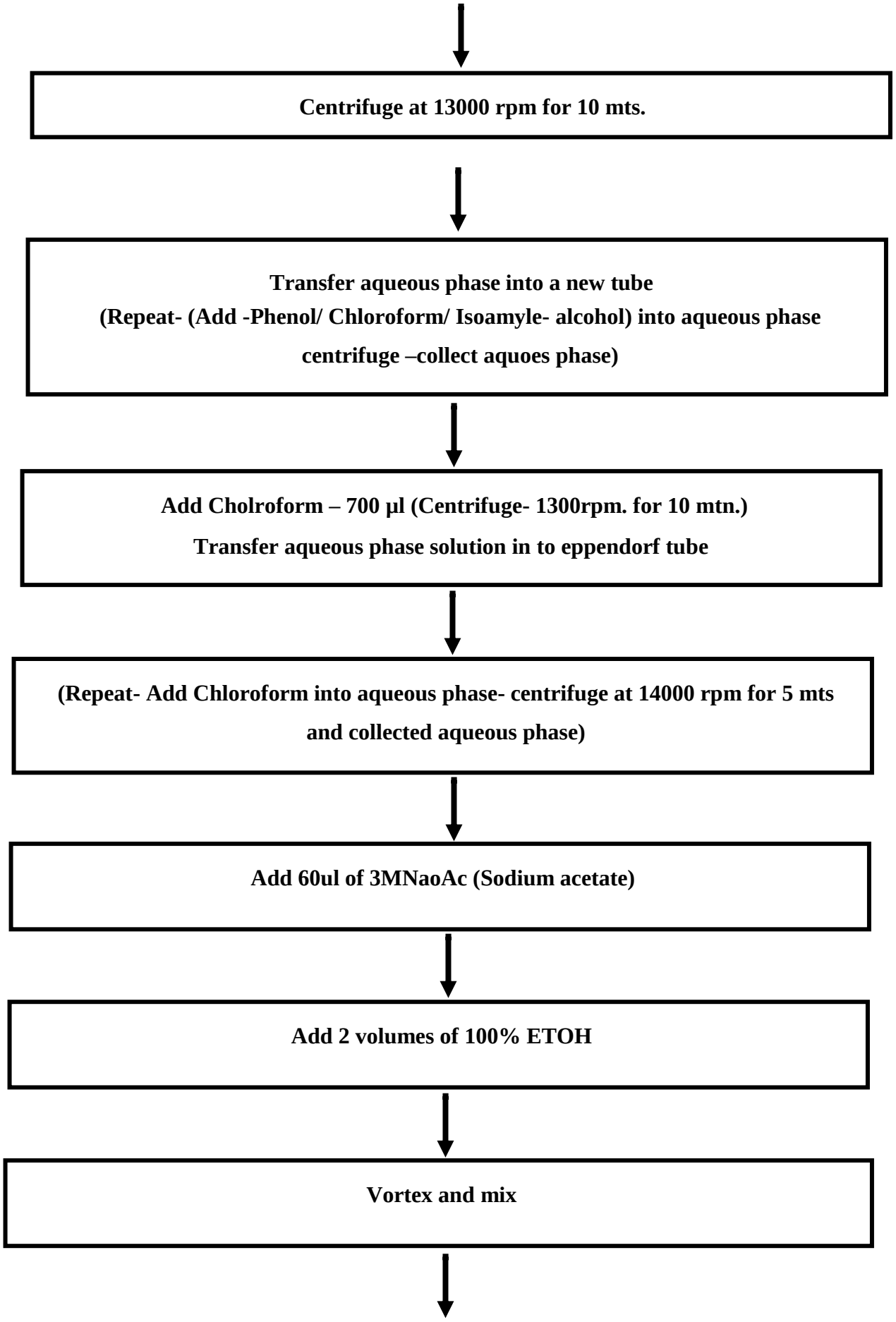
Parasites were collected from different slaughtered houses Lucknow (Uttar Pradesh) and are preserved in 70% alcohol.

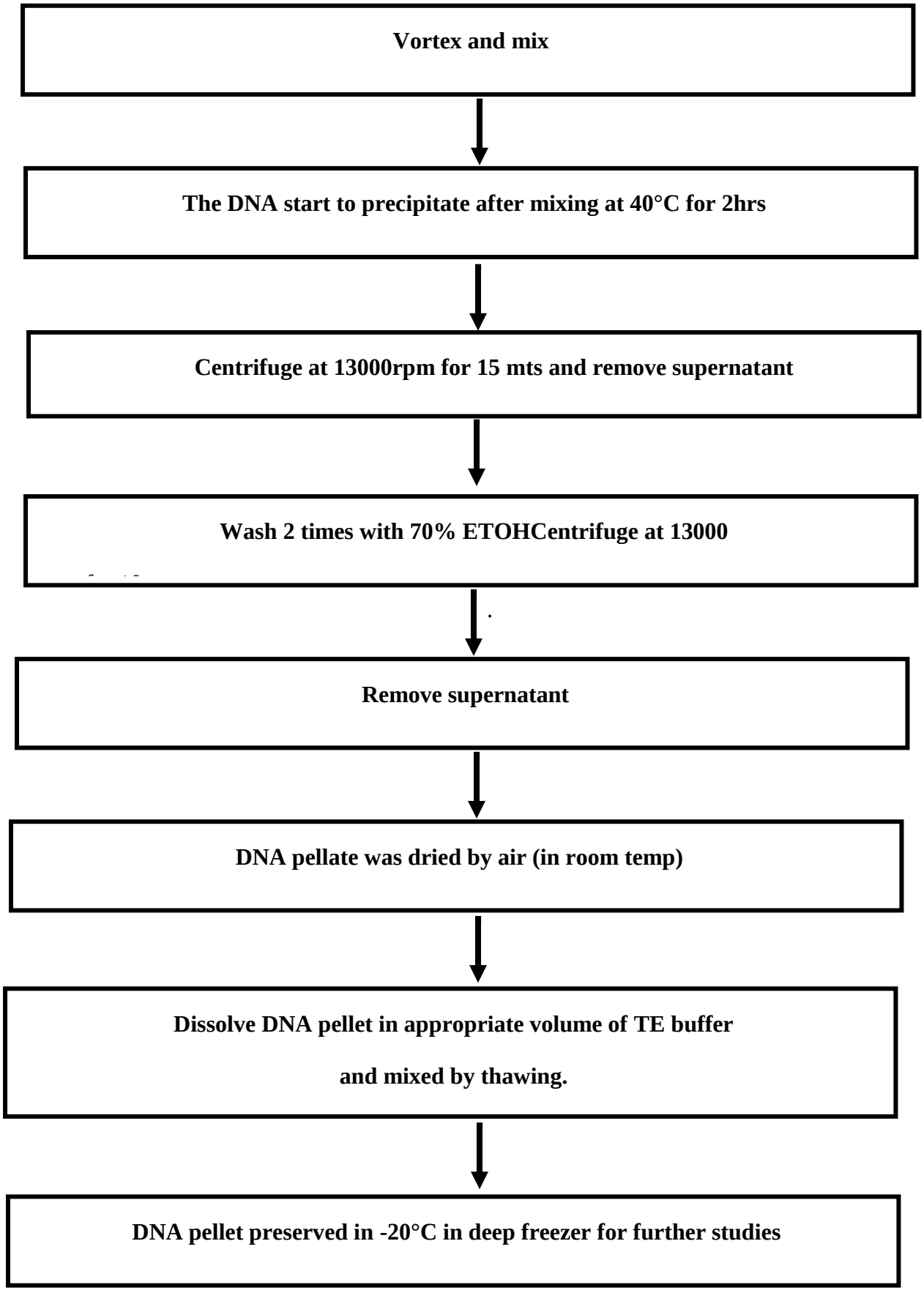
### 5.2. 2 DNA extraction

DNA extraction was done by P:C:I method (Phenol/chloroform/isoamyl alcohol) which is composed by Harris *et al*., 1999 and cornman *et al*., 2009. The protocol of DNA extraction as under using genomic mannual method (PCI) was followed.




Collection of pellets and discard the supernatant added 700 µl Lysis buffer in the tube
Add 10 µl RNase A
Incubate at 37 0°C for 2 hours
Add 10 µl protenase k
The solution was left overnight
Further solutions after lysis treatment were vortex and centrifuge at 14000 rpm for 2 mts.
Supernetent transfer to new tube and add equal ratio of Phenol/chloroform/isoamyl alcohol (P:C:I) in the ratio of (25:24:1)
Mix well


Centrifuge at 13000 rpm for 10 mts.
Transfer aqueous phase into a new tube
(Repeat- (Add -Phenol/ Chloroform/ Isoamyle- alcohol) into aqueous phase centrifuge –collect aquoes phase)
Add Cholroform – 700 µl (Centrifuge- 1300rpm. for 10 mtn.)
Transfer aqueous phase solution in to eppendorf tube
(Repeat- Add Chloroform into aqueous phase- centrifuge at 14000 rpm for 5 mts and collected aqueous phase)
Add 60ul of 3MNaoAc (Sodium acetate)
Add 2 volumes of 100% ETOH
Vortex and mix


Vortex and mix
The DNA start to precipitate after mixing at 40°C for 2hrs
Centrifuge at 13000rpm for 15 mts and remove supernatant
Wash 2 times with 70% ETOHCentrifuge at 13000
Remove supernatant
DNA pellate was dried by air (in room temp)
Dissolve DNA pellet in appropriate volume of TE buffer
and mixed by thawing.
DNA pellet preserved in -20°C in deep freezer for further studies

### 5.2.3 Agarose Gel Electrophoresis for Quantification and Quality Analysis of DNA

This method is used for the analysis of quality and quantification of DNA which is based on the Etbr (ethidium bromide fluorescent) staining of DNA. The quantity of DNA can be predictable by fluorescent yield of the samples and provide good quality of the DNA.

**Procedure.**

Preparation of 0.8% agarose gel (0.8 g agarose powder)

- Agarose powder dissolved in 100ml of 1XTAE buffer (Make from 50X TAE bufffer).
- Add 12µl Etbr and poured in the casting tray with comb of electrophoresis unit. When gel is solidified, transferred to electrophoresis unit filled with 1X TAE buffer.
- Mix with 1 µl loading dye to 6 µl of DNA sample loading in the wells of the gel.
- DNA Samples were migrated at the voltage of 60V in the gel electrophoresis.
- Migrate the sample on the gel till the dye has run one third of the distance in the gel.
- DNA band was visualized using under UV (GeNei TM) at 254nm and the band in assessment with the fluorescent acquiesce of the standards.

### 5.2.4 DNA quantification

Quantification of Genomic DNA were Isolated from desired sample done by spectrophotometer (Biophotometer D-30) and few samples were also detected by 0.8% agarose gel electrophoresis unit. Quantification of nucleic acids is an accurate and simple view of the concentration of DNA sample. The ratio of OD260/OD280 should be used checking the purity and quantity of DNA.

Procedure of spectrophotometer reading -

- Take 1000 µl TE buffer in a cuvette and standardize the spectrophotometer at 260nm as well as 280nm.

- Add 01 µl of DNA sample to mix in 99 µl TE (Tris-EDTA buffer) and mix well.
- TE buffer was used blank in the other cuvette of the spectrophotometer.
- Calculate the OD260/OD280 ratio.
- The amount of DNA can be quantified using the formula:

$$\text{DNA Concentration (µg/ml)} = \frac{OD260 \times 100\ (Dilusion\ factor) \times 50 µg/ml}{1000}$$

- DNA ratio 1.8-2.0.

### 5.2.5 PCR Programme

PCR (Polimerase Chain Reaction) product was amplifying with the help of Internal Transcribed Spacer 2 (ITS2) marker for the identification of three nematode parasitic genomes. PCR was setup for preparation of Genomic DNA to need the volume of 25 µl of sample mixture. The final 25µl reaction volume was set up using 10 µl TaqPCR, Mastermix, 2.0 µl of forward primer, 2.0 µl reverse primer, 8.0 µl DNA, 3.0. PCR reagents were used for the purposes of optimization.

### 5.3.5 PCR Reaction set-up Table

**5.1- PCR setup of volume**

| PCR Components | Volume(µl) |
|---|---|
| DNA | 08 |
| ddH2O | 03 |
| Forward primer | 2 |
| Reverse primer | 2 |
| PCR Master Mix | 10 |
| Total Reaction Volume | 25 |

**PCR conditions**

The PCR reaction was performed according to the PCR condition. PCR set up conclude the reaction was conducted based on denaturation, optimal temperature of annealing and extension. Reaction conditions depend on the denaturation temperature necessary to activate the enzyme in each master mix. The amplification concerned an initial denaturation of 94°C -95°C for maximum of 1mtn. Annealing temperatures ranging from 54°C-62°C were tested and sample was optimised with a final extension at 72°C for 10 mts.

**Table 5.2- PCR conditions setup of volume**

| | **Temperature (°C)** | | | **Time** | | |
|---|---|---|---|---|---|---|
| | (A) | (B) | (C) | (A) | (B) | (C) |
| Initial Denaturation | 95 | 94 | 94 | 50s | 3m | 1m |
| Final Denaturation | 95 | 94 | 94 | 30s | 1m | 1m |
| Annealing | 55 | 58 | 54 | 35 s | 1m | 1m |
| Extension | 72 | 74 | 72 | 45 s | 1m | 2m |
| Final Extension | 72 | 74 | 72 | 10 m | 10m | 7m |
| Cooloing | 4 | 4 | 4 | - | - | - |

**5.2.6 PRIMERS**

Three sets (Forward and Reverse) of primers of nematode parasites species were considered for PCR amplification.

(A) Reported Primer by Ahmad *et al*., (2015) (ITS2) of *Haemonchus* sp.

F- ACGTCTGGTTCAGGGTT

R-TTAGTTTCTTTTCCTCCG

(B) *Trichuris* sp. parasites primer was self designed by self with help of the NCBI-Fasta format – BLAST – Clustral W-Primer 3plus and Oligo analyzer from different Accession no. –LN813018.1, AJ238220.1, FR87027741.1 etc…

F-GTCGTCCTAAGCAGGAGTCG

R-TCATTGCCGTAAACCAACAA

(c) Reported Primer by Bandyopadhyay *et al.,* (2009).

F-CATTGCAACATGCACTATG

R-ACAGTTGTCATACAGGCCCC.

### 5.2.7 Methods for PCR amplification

- Electrophoresis of amplified DNA fragment was conducted in 2% agrose gel in 1x TBE buffer and stained with ethidium bromide (Etbr) to verify the bands. These bands compared to the 1kb DNA ladder.
- PCR product band size was checked under UV transilluminator and taken image from the gel doc.
- The PCR product has been purified by the PCR purification kit.

**Procedure:**

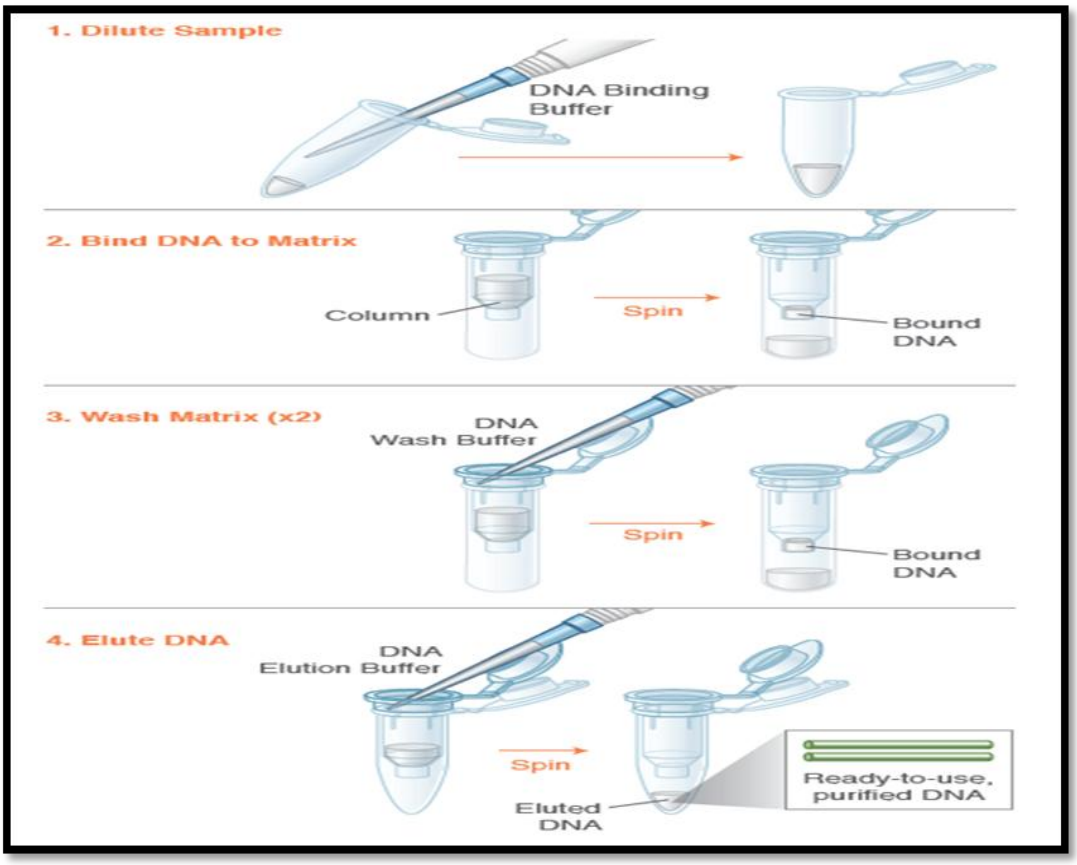



**Figure 5.1 Purification of DNA by elution kit (Purificattion kit)**

**Source:** https://www.neb.com/products/t1030-monarch-pcr-dna-cleanup-kit-5-ug

**5.2.8 Sequencing:** The pure PCR product was sent for sequencing (Progen Biolab and Rahul Scientific Traders).

### 5.2.9 Phylogenetic Tree

- Sequences generated after sequencing BLAST (Basic Local Alignment Search Tool, NCBI) was performed and aligned for knowing homology and edited using MEGA software version 6 (Tamura *et al*., 2011) in both directions (forward and reverse sequences).
- Clastal-W is aligned and sequenced.
- The sequence identity computed using the BioEdit (Hall, 1999) and compared with the reference sequences of each nematode parasites species with accession number.
- Phylogenetic analysis was conducted using neighbour joining method
- The phylogenetic tree analysis was conducted using the (UPGMA), distance method based on Neighbour joining method by by MEGA7.0 software (Tamura *et al*., 2011).

## 5.3 RESULTS

This investigation revealed that the nematode parasites species (*Haemonchus contortus, Trichuris ovis* and *Oesophagostomum columbianum*) by molecular technique (PCR, DNA sequencing and bioinformatics). The amplified PCR product range of approximately 325bp, 205bp and 117bp (Figure no-5.3 and 5.4). The generated sequence was blasted (BLAST) in nucleotide and compared to different accession number which showed homology (97.50% to 100%), is identified species of all nematodes after using bioinformatics methods (BLAST, Clastal W, Oligo analyzer and Mega 7.0 software) generating sequence. The phylogenetic tree analysis showed relationship between various species of parasites and represented by phylogeny tree.

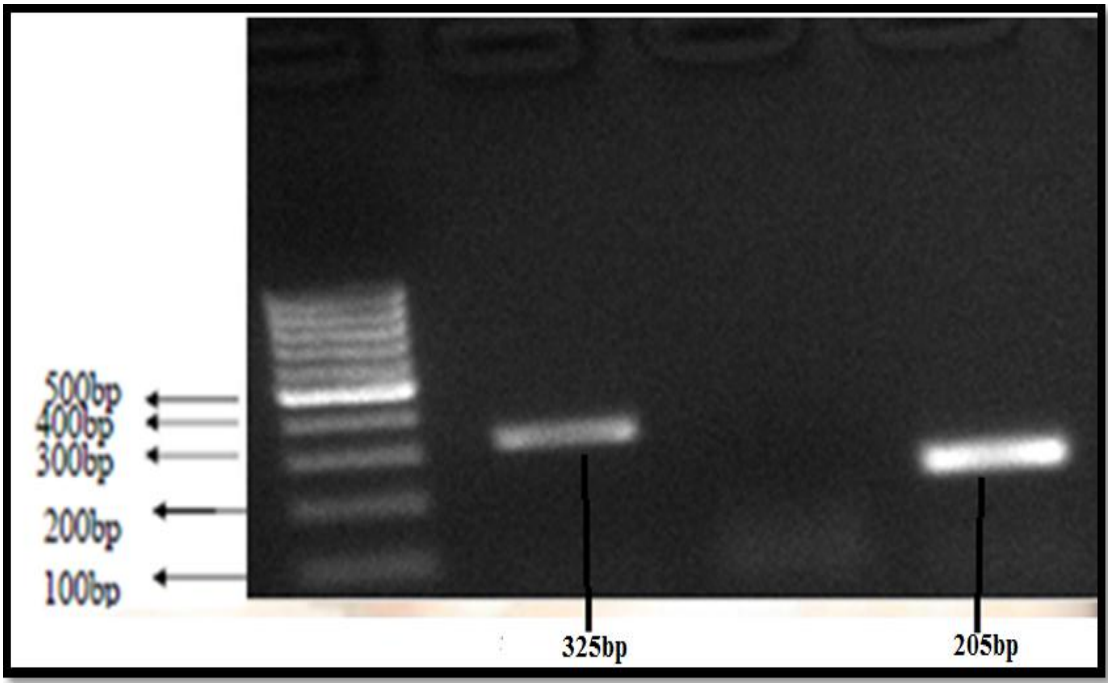



**Figure 5.2 PCR purified product size 325bp and 205bp, loaded with 1 kb DNA ladder. 2 % agarose gel showing amplified DNA of *Haemonchus and Trichuris* species**

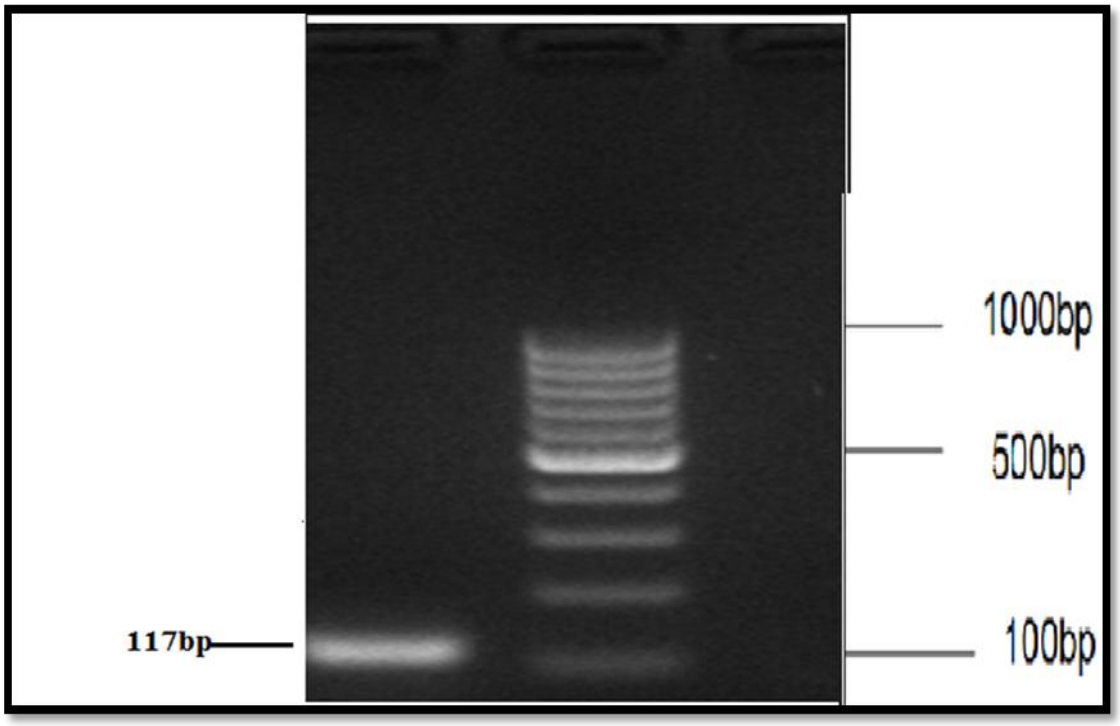



**Figure 5.3 PCR purified product size 117bp loaded with 1 kb DNA ladder. 2% agarose gel showing amplified DNA of *Oesophagostomum* species**

### 5.3.1 Sequencing

The amplified product was purified by the purification kit and PCR product was send for sequencing.

### 5.3.2 Phylogenetic analysis

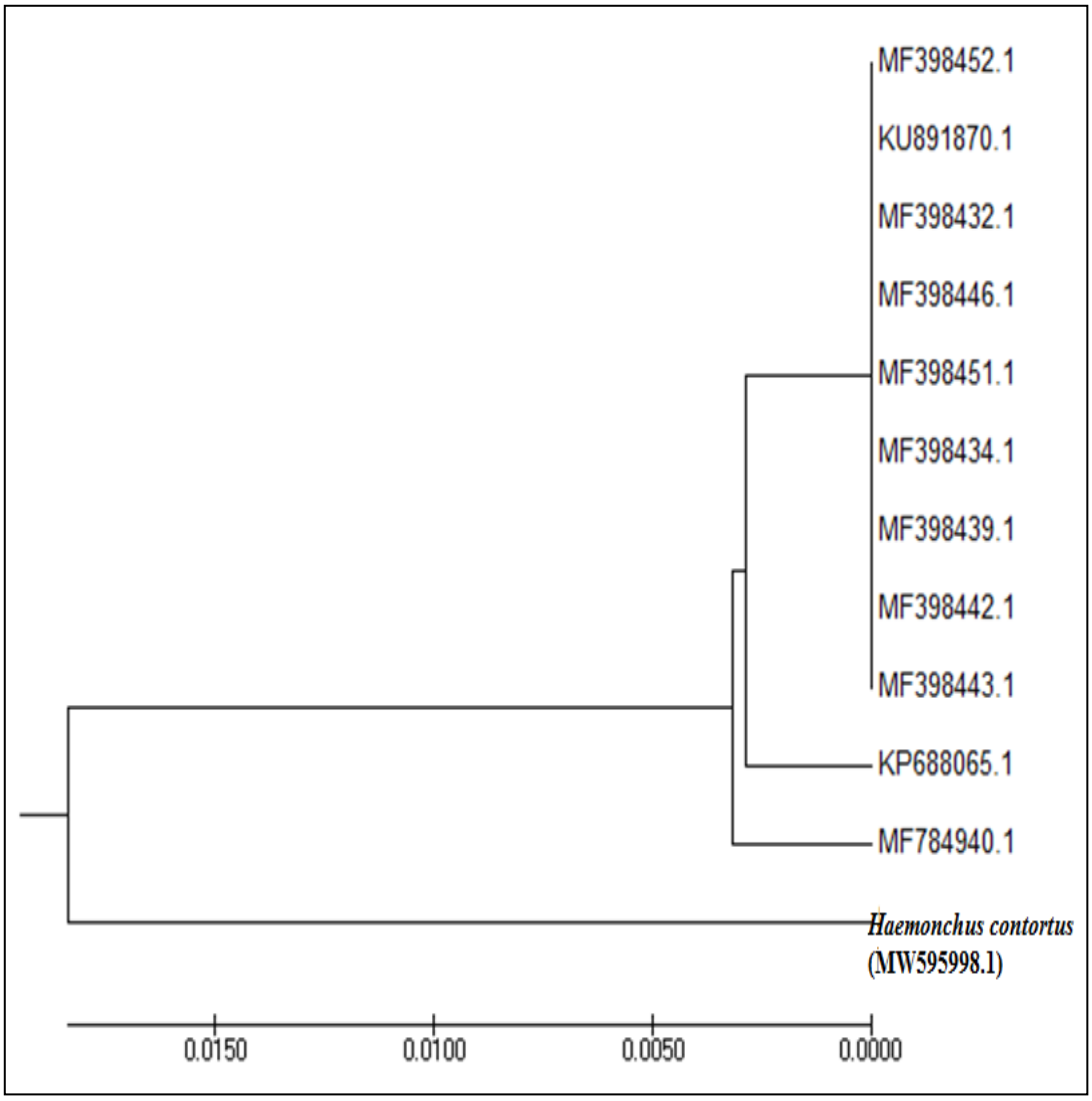



**Figure 5.4 Phylogeny tree analysis was constructed by using UPGMA method. Accession no. for each sequence is given on the tree. The tree has been represented homology and evolutionary distances with *Haemonchus contortus***

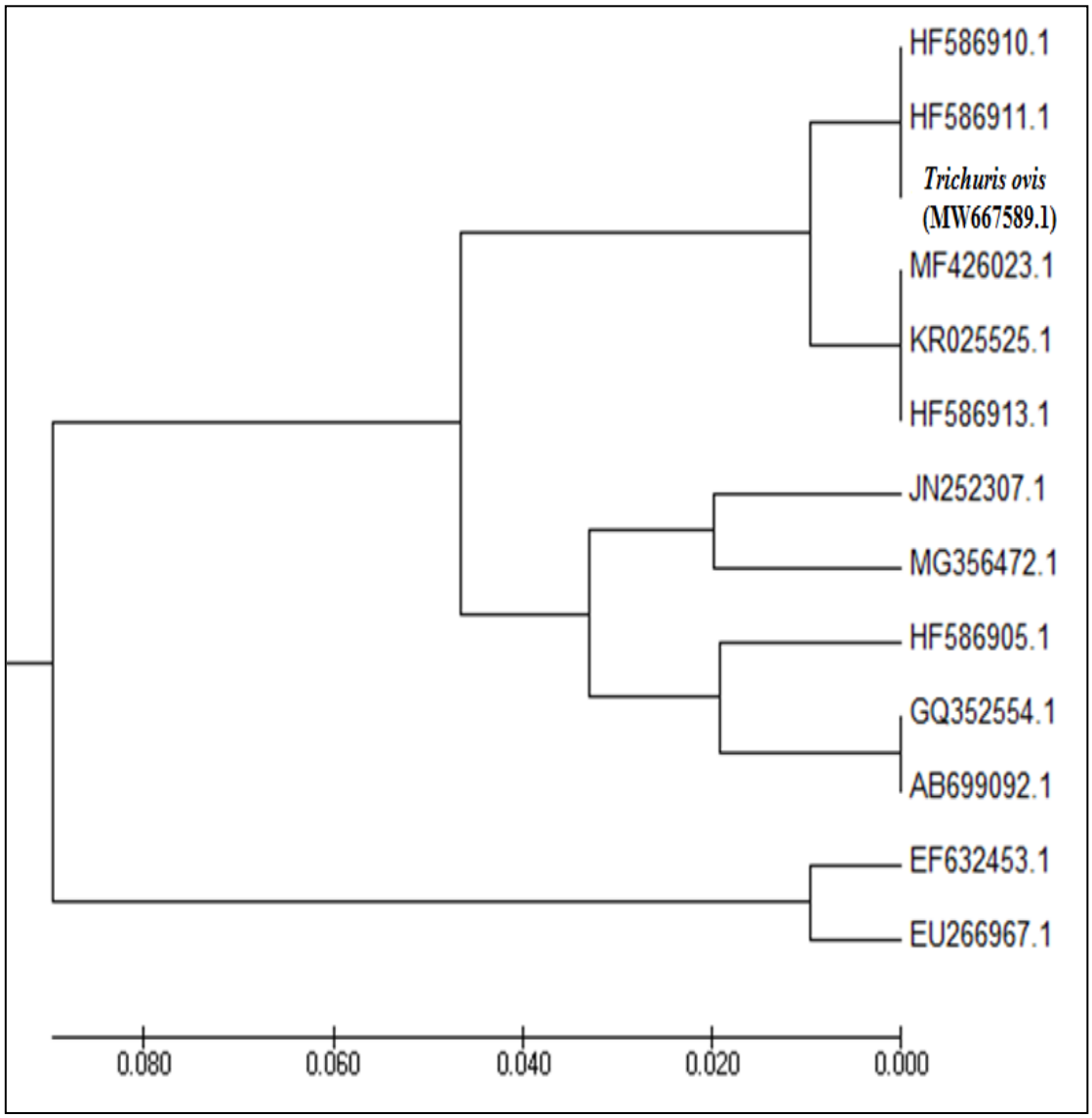



**Figure 5.5 Phylogeny tree analysis was constructed by using UPGMA method. Accession no. for each sequence is given on the tree. The tree has been represented homology and evolutionary distances with the *Trichuris ovis***

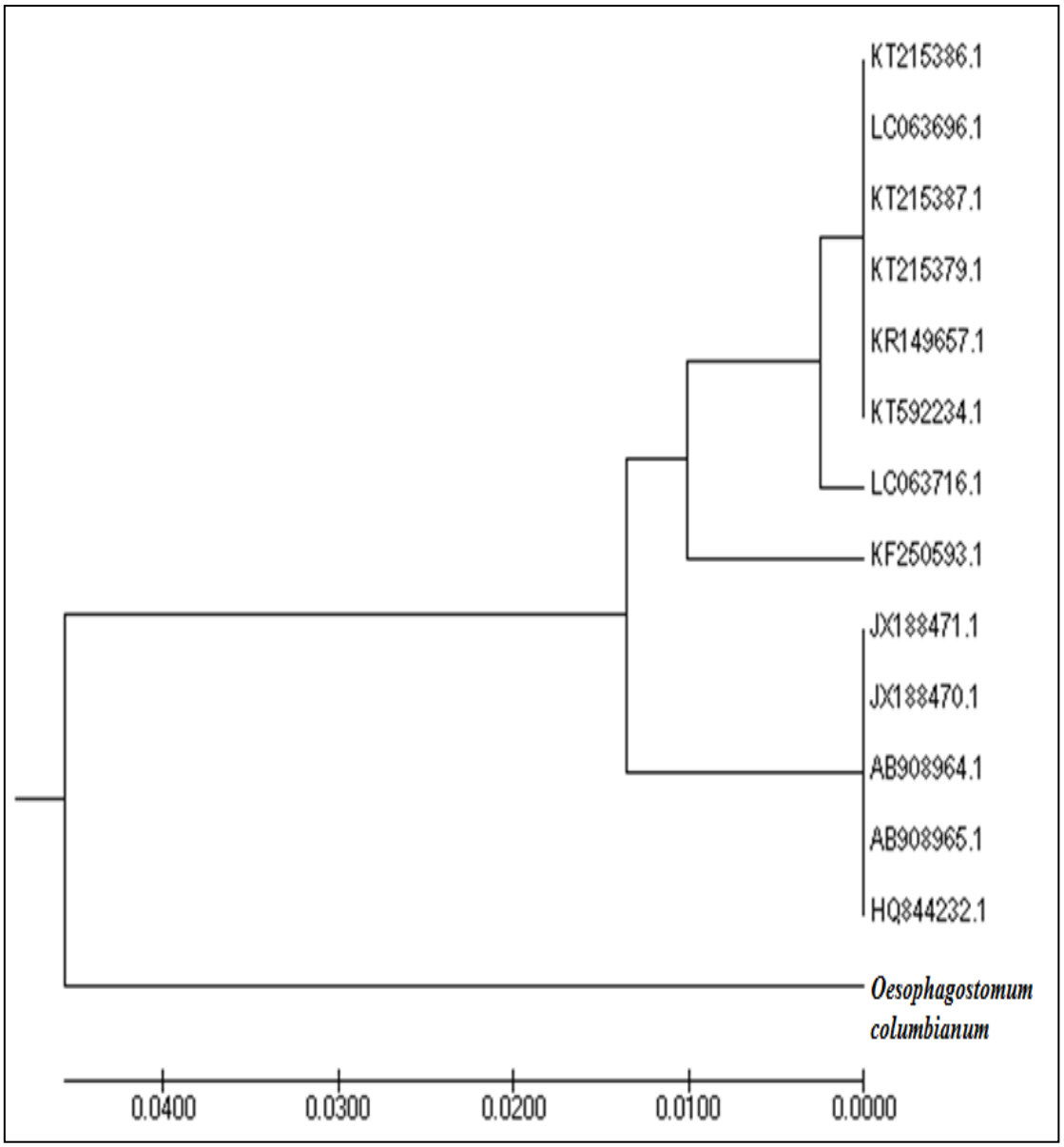



**Figure 5.6 Phylogeny tree analysis was constructed by using UPGMA method. Accession no. for each sequence is given on the tree. The tree has been represented homology and evolutionary distances with the *Oesophagostomum columbianum***

### 5.3.3 DNA extraction and PCR amplification analysis

Genomic DNA of gastrointestinal nematode parasites of goat was elaborated by molecular techniques. The molecular characterization techniques are utilized for the characterization and identification of specific Genomic studying of nematode parasites. These techniques have become widely used in the world (Dahlgren *et al*.,

2010). The study was conducted to extract the genomic DNA from nematode parasites with the standard method of PCI (Phenol: chloroform: Isoamyl alcohol) (Mabe, 2012). Extracted DNA was quantifyed by Spectrophotometer and Gel electrophoresis unit and observed the high intensity of band. The PCR product was amplified from the genomic DNA of *Haemonchus* sp., *Trichuris* sp. and *Oesophagostomum* sp. PCR amplification products have yielded 325bp and 205bp and 117bp fragment in size. Three sets of primer targeting the Internal Transcribed Spacer 2 (ITS2) for genomic-specific were successfully amplified and sequenced.

### 5.3.4 Sequencing

The amplified product was purified by the purification kit and PCR product was sent for sequencing. The PCR amplified product size of approximately 90 to 325bp (H*aemonchus* sp. *(*325bp*), Oesophagostomum* sp. (117bp) and *Trichuris* sp. (205bp) (Figure 5.2 and 5.3) were confirmed by the genome of parasitic species by checking sequences size.

### 5.3.5 Sequence generated after sequencing of PCR product

Sequence of *Haemonchus* sp. (Partial sequence of conserverd region).

TACGTCTGGTTTCAGGGTTGTTAACCATATACTACAATGTGGGTAA
TTTCAACATTGTTTGTCAAATGGCATTTGTCTTTTAGACAATTCCCATTTC
AGTTCAAGAACATATACATGCAACGTGATGTTATGAAATTGTAACATTCC
TGAATGATATGAACATGTTGCCACTATTTGAGTGTACTCAGCGAATATTG
AGATTGACTTAGATAGTGACTTGTATGGCGACGATGTTCTTTTATCATTT
GTATAATGCAACACTGAGCTCAGGCGTGATTACCCGCTGAACTTAAGCAT
ATCATTTAGCGGAGGAAAAGAAACTAAA

Sequence of *Trichuris* sp. (Partial sequence of conserverd region)

TGTCGTCCTAAGCAGGAGTCGTTCCGTCGCTCGTCACCTGTTTGATCAAG
ATCGTCCTGGATGCTCTTCGGTGAGTGTCCTTGGCGACTTGAACGTTTAC
TTTGAGAAAATGGAAGCGCTCAAGGCAGGCCGTAGAGCTTGAACAGTGG
TGCATGGAATAATGAAAGATGGCCTCGGTGCTATTTTGTTGGTTTACGGC
AATGAA

Sequence of *Oesophagostomum* sp. (Partial sequence of conserverd region)

CACCTTGGGGTCGGTATGGTCGTGACCTCGTTGTCACTGTCAAAGC GTTTAGCGACTAAGAATGCTTTGGCGGGGCCTGTATGACAACTGTA

### 5.3.6 Phylogeny tree analysis

DNA sequences were edited using MEGA 7.0 software (Kumar *et al*., 2016). The generated sequences of *Haemonchus* sp., *Trichuries* sp. and *Oesphagostomum* sp. were submitted to GenBank with an accession number. Multiple sequence alignments data were analyzed by Clustal W and GenBank database in the BioEdit program (Hall, 1999). The phylogenetic tree was constructed with the help of UPGMA method in the MEGA 7.0 programs. The evolutionary distances were computed using the Kimura 2-parameter method.

The sequences were aligned and compared with the ITS2 sequences of the *Haemonchus* sp. from the GenBank database, using NCBI and BLAST tool are used for the similarity determination. Alignment of sequences revealed 99.07% similarity with generated accession no. MW595998.1 of *Haemonchus contortus* (Accession no. -MF398451.1, MF398452.1, MF398446.1, MF398443.1, MF398439.1, MF398442.1 and MF398434.1). The second generated sequence of amplified ITS-2 sequences number MW667589.1 of *Trichuris* sp. revealed that 100% similarity with *Trichuris ovis* (Accession no.- HS586910.1, HF586911.1, KR025525.1, and JN252307.1). Third sequence was of *Oesophagostomum* sp. was revealed that 97.50% similarity with *Oesophagustomum columbianum* (Accession no.-AB908965.1, AB908964.1, JX188471.1, KT215387.1 and HQ844232.1).

## 5.4 DISCUSSION

Molecular is very advanced technique, which is highly senstive, enabled the accurate, widely used for identification and diagnosis of gastrointestinal parasitic infection (Dahlgren *et al.*, 2010; Dahlgren *et al.*, 2010; Rosenthal *et al*., 2008 and Dangoudoubiyam *et. al.*, 2009). PCR (Polymerase Chain Reaction), DNA sequencing and Bioinformatics are the most important tools in my study (Gasser *et al*., 1993 and Sweeny *et al.*, 2011). Ghai *et al.*, (2014) have reported that PCR technique is most sensitive than microscopy for analysis of parasitic species.

Abbaszadegan *et al.*, 2007, reported that PCR is used as analytical methods and observed the genetic variability between the parasites at genomic and species-specific level. rDNA sequences are most important molecular markers used for differentiation of parasites (Rosenthal *et al.*, 2008 and Xiang *et al.*, 2009). Internal Transcribed Spacer 2 (ITS2) of nuclear rDNA (Ribosomal Deoxyribonucleic acid) sequences is most important reliable genetic marker used for species identification (Campbell *et al*., 1995; Stevenson *et al*., 1995; Gasser, 2006; Gasser *et al*., 1993; Gharamah *et al*., 2012 and Xiang *et al.*, 2009) due to the high inter-specific sequence difference and intra-specific sequence correlation. It is very useful tool for species specific identification of the parasites (Epe *et al*., 1997; Heise *et al*., 1999; Luton *et al*., 1992, Hoste *et al*., 1995 and Bott *et al*. 2009) ITS2 DNA region are species specific primers of *H. contortus* and *Trichostrongylus* spp. in a livestock.

However, the genetic variability of Internal transcriber spacer 2 (ITS2) within depending on the parasite population studied (Gasser and Newton, 2000 and Gasser, 2006). Sequence analyses of the multicopy of ribosomal DNA (rDNA) encoding of genes and more conservative primers has binding to the rDNA of many nematode parasites (Heise *et al.*, 1999) and phylogenetic tree analysis (Cerutti *et al*., 2010). Phylogenetic tree analysis of the ITS-2 sequences was most reliable genetic marker for the identification of nematode parasites (*Haemonchus contortus*, *Oesophagostomum columbianum* and *Trichuri ovis*) they were constructed by UPGMA method in the MEGA7 program (Kimura, 1980, Saitou, 1987; Chilton *et al*, 1995; Tamura *et al.*, 2007; Lin *et al.*, 2012; Ota *et al.*, 2015, Callejón *et al.*, 2013 and Kumar *et al.*, 2018).

Molecular characterization is one of the most common and crucial for identification of species, which have a broad range of utilization (Criscione, 2005 and Gasser, 2006). This study provided the first data of the ITS2 (Internal Transcribe spacer 2) region derived from 325bp amplifying sequence of *Haemonchus contortus* from gastrointestinal parasites of goat in different areas of Lucknow (Uttar Pradesh). This data was based on findings of Ahmad *et al.,* (2015), Hassan *et al.,* (2017) and Stevenson *et al*. (1995). ITS2 region for identification of *Haemonchus contortus*, which is widely used by many authors (Blouin, 199, Bensch *et al*., 2004, Chilton *et al*., 2001, Troell *et al*., 2003, Brasil *et al*., 2012) in analyzing sample from many

parts of the world. Similarly, a high diversity of ITS2 sequences was observed with 98 to 100 homologies in *Haemonchus contortus* sequences from the GenBank database in goats, sheep, and cattle. (Akkari *et al*., 2013 and Mangkit, *et al*., 2014). Fakae and Chiejina (1993) also reported significant higher variation and 90% to 100%, homology depicted in species of *Haemonchus contortus* in gastrointestinal tract of goats (Tiong K Tan *et. al.*, 2014). Ahmed *et al*., 2015 suggested that alignment of ITS-2 sequences at 187,196 and 208 nucleotide and Phylogenetic tree analysis was showed using Neighbour-Joining method, 98-100% homology. Abramatov *et al*., 2013 have also reported similar study.

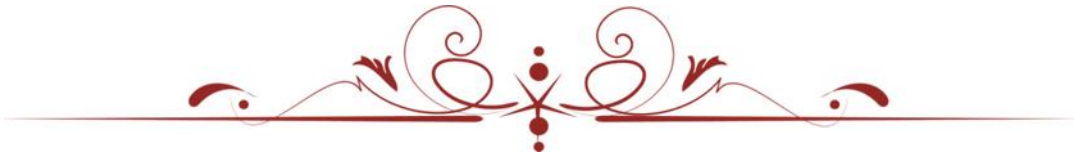
The genus of *Trichuris was* recognized by Roederer 1761. This is very common nematode parasites which are found in the gastrointestinal tract in the goat. In this study, it was found that 205bp binding sized of *Trichuris ovis* and phylogeny tree analysis homology showed that 100% similar studies were found in the paper of Bandyopadhyay *et al*., (2009), Abildgaard, 1795 and Misal, *et al.,* 2015. Many research articles related to other species, *Trichuris skarjabini*, *Trichuris discolor* and *Trichuris leporis* (Baskakaw, 1924, Linstow, 1906 and Froelich, 1789). Liu *et al*., 2014 and Cutillas *et al*., 2009 have also suggested the genomic study of *T. ovis* and *T. discolor*, and detected a substantial difference.

Bandyopadhyay *et al.*, 2009 reported similar study on the ITS-2 region used for identification of different species of *Oesphagostomum* (nodular worm) parasite (*O. columbianum* and *O. venulosum*,). Sweeny *et al.*, (2011) have reported the PCR technique based study. Kimura, (1980) also reported that the phylogenetic tree of the ITS-2 sequences was constructed by UPGMA method in the MEGA7 program (Saitou, 1987; Chilton *et al*, 1995) Many scientists also reported similar findings (Tamura *et al.*, 2007, 2011, Lin *et al.*, 2011; Ota *et al.*, 2015 and Kumar *et al.*, 2018).

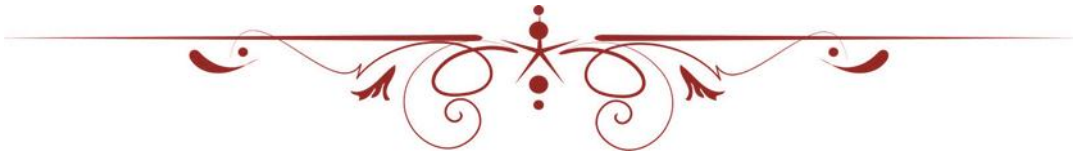
**5.4 Conclusion**

The present study was based on the molecular characterization of nematode parasites. This study highlights the integrated study of detection of nematode parasites and allowing a more complete understanding of the genetic variation, bioinformatics, and long evolutionary process by sequences and phylogeny tree analysis. The findings of this study revealed that the data was amplified the product

band size of gastrointestinal nematode parasites (*Haemonchus* (325bp-MW595998.1), *Oesophagostomum* (117bp) and *Trichuris* species (205bp-MW667589.1). ITS-2 region was using rDNA interspecific difference and showed genetic relationship. The online blasting (BLAST) revealed that the nucleotide sequence has finding significant homology with nematode parasites species and higher score homology which showed 100% were obtained with *Trichuris ovis* species following to another species of parasites, *Haemonchus contortus* (99.07%) and *Oesophagostomum columbianum* (97.50%). The study provided the molecular evidence of parasitic infection in Lucknow region. Molecular method having rapid changes in progress of research field with in significant genetic diversity. This observation might have implications for further study epidemiology, diagnosis of parasitic infection and control program.



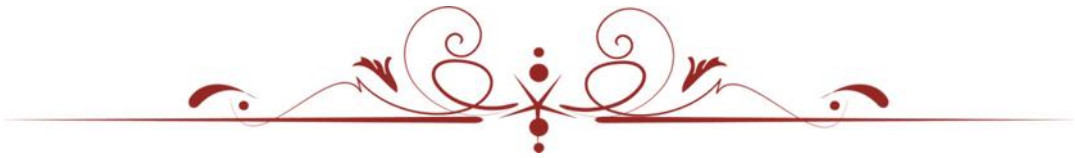
# Chapter-6
# Significance and future prospects



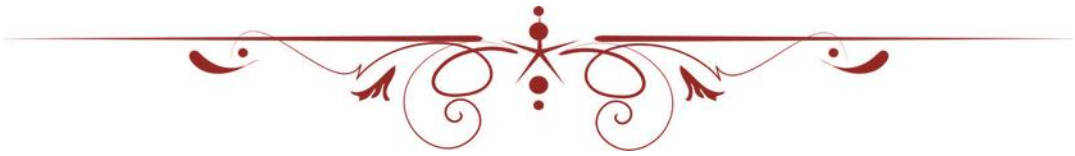
# CHAPTER-6
# SIGNIFICANCE AND FUTURE PROSPECTS

Focus of major researches in India and Worldwide has been the examination, biology and epidemiological studies of gastrointestinal nematode parasites. A significant aspect of studying the prevalence, distribution, and seasonal patterns of transmission of disease in different climatic zones and improving treatment and control is taken up in the current research. Epidemiological data obtained by tools would help authorities and Food and Agricultural Organization (FAO), in the detailed mapping of the prevalence and by this method can better understand parasitic infection and abundance. Molecular characterization is a specific diagnosis method to identify nematode infections for refined investigations of parasite epidemiology.

In future, these methods, and high-performance field-based assays and sequencing technologies provide better prediction for disease surveillance and control programs against nematodiasis. Provide greater protection for the consumer of animal products through reduced anthelmintic use and improvement of grazing management where tethering is in practice could reduce the risk of parasitic infection. Immunology and molecular based technique can be widely used for understanding the helminth parasites.



# *References*



# REFERENCES


Abebe, G., Dawson, L. J., Detweiler, G., Gipson, T. A., Sahlu, T., Merkel, R. C., & Goetsch, A. L. (2000). The opportunities and challenges of enhancing goat production in East Africa. In A conference held at Awassa College of Agriculture, Debub University (pp. 10-12).

Abebe, W. and G. Esayas, (2001). Survey on ovine and caprine gastro-intestinal helminthosis in eastern part of Ethiopia during the dry season of the year. Revue Vet. Med., 152(5): 379-384.

Abramatov, M. B., Amirov, O. O., Euchboev, A. E., Khalilov, I. M., & Abdurakhmanov, I. Y. (2013). Morphological and molecular characterization of Haemonchus contortus and H. placei (Nematoda: Trichostrongylidae) from Uzbekistan by sequences of the second internal transcribed spacer of ribosomal DNA. *Sci Parasitol*, *14*, 115-120.



Abramatov, M. B., Amirov, O. O., Euchboev, A. E., Khalilov, I. M., & Abdurakhmanov, I. Y. (2013). Morphological and molecular characterization of Haemonchus contortus and H. placei (Nematoda: Trichostrongylidae) from Uzbekistan by sequences of the second internal transcribed spacer of ribosomal DNA. *Sci Parasitol*, *14*, 115-120.



Abubakar, U. (2002). The incidence of liver condemnation due to fascioliais and its economic implications in Zaria abattoir DVM thesis. *submitted to the department of veterinary parasitology and entomology, faculty of veterinary medicine, ABU Zaria*.

Achi, Y. L., Zinsstag, J., Yao, K., Yeo, N., Dorchies, P., & Jacquiet, P. (2003). Host specificity of Haemonchus spp. for domestic ruminants in the savanna in northern Ivory Coast. *Veterinary parasitology*, *116*(2), 151-158.

Aga, T. S., Tolossa, Y. H., & Terefe, G. (2013). Epidemiology of gastrointestinal nematodes of Horro sheep in Western Oromiya, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, *5*(10), 296-304.

Agyei, A. D. (1991). Epidemiological observations on helminth infections of calves in southern Ghana. *Tropical animal health and production*, *23*(3), 134-140.

Ahmed M, Singh M, Bera A, Bandyopadhyay S and Bhattacharya D (2011). Molecular basis for identification of species/isolates of gastrointestinal nematode parasites. *Asian Pacific Journal of Tropical Medicine,* 4 (8), 589-593.

Ahmed, I. I. B., Ahmed, M. A., Ahmed, A. A., & Mohamed, F. (2015). Morphological and molecular characterization of Haemonchus contortus (Rudolphi, 1803) Cobb, 1898 (Nematoda: Trichostrongyloidea) from sheep, Ovis aries in Egypt based on the second internal transcribed spacer of ribosomal DNA. *International Journal of Advanced Research*, *3*(5), 1152-1161.

Ahmed, J., Duguma, A., Regassa, D., Belina, D., & Jilo, R. (2017). Gastrointestinal nematode parasites of small ruminants and anthelmintics efficacy test in sheep of Haramaya district, eastern Ethiopia. *Anim Vet Sci*, *5*(3), 39-44.

Ahmed, M., Singh, M. N., Bera, A. K., Bandyopadhyay, S., & Bhattacharya, D. (2011). Molecular basis for identification of species/isolates of gastrointestinal nematode parasites. *Asian Pacific Journal of Tropical Medicine*, *4*(8), 589-593.

Ahmed, N. (1988). Prevalence of GIT helminthes and the comparative efficiency test of nematocidal drugs in goats of Wallaga administrative region at Mechara settlement area. *DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit, Ethiopia*.

Akhter, N., Arijo, A. G., Phulan, M. S., Iqbal, Z., & Mirbahar, K. B. (2011). Prevalence of gastro-intestinal nematodes in goats in Hyderabad and Adjoining Areas. *Pak Vet J*, *31*(4), 287-290.

Akkari, H., Gharbi, M., Awadi, S., Mohamed, A. D., & Kumsa, B. (2013). New sublinguiform vulvar flap of Haemonchus species in naturally infected domestic ruminants in Béja Abattoir, North Tunisia. *Veterinarski arhiv*, *83*(3), 281-291.

Alam, J., (1993). Livestock: The sector for more investment in Bangladesh. Asian Livestock (7): 778.

Aliu, S. K., Joseph, D. H., & Abbagana, S. (2001). Epidemiological studies of gastro intestinal parasitic infection in northern eastern zone of Nigeria. *Vet. Rec*, *187*, 268-279.

Allonby, E. W. and Urquhart, G. M., (1975). The epidemiology and pathogenic significance of haemonchosis in a merino flock in East Africa. *Veterinary Parasitology*, 1, 129-143.

Al-Quaisy, H. H. K., Al-Zubaidy, A. J., Altaif, K. I., & Makkawi, T. A. (1987). The pathogenicity of haemonchosis in sheep and goats in Iraq: 1. Clinical, parasitological and haematological findings. *Veterinary parasitology*, *24(3-4)*, *221-228.*

Al-Shaibani, I. R. M., Phulan, M. S., Arijo, A., & Qureshi, T. A. (2008). Epidemiology of ovine gastrointestinal nematodes in Hyderabad district, Pakistan. *Pakistan Veterinary Journal*, *28*(3).

Alvarado-Villalobos, M. A., Cringoli, G., Maurelli, M. P., Cambou, A., Rinaldi, L., Barbachano-Guerrero, A., & Serio-Silva, J. C. (2017). Flotation techniques (FLOTAC and mini-FLOTAC) for detecting gastrointestinal parasites in howler monkeys. *Parasites & vectors*, *10*(1), 586.

Amenu, A. (2005). Epidemiology of gastrointestinal tract nematodiosis of small ruminant in three different agroecological zones of southern Ethiopia. *Master of Science Thesis in Tropical Veterinary Epidemiology, School of Graduate Studies, Addis Ababa University*, 86.

Amulya, G., Sudharani, R., Ismail, M., Shareef, & Gopinath, S.M. (2015) Anthelmintics and anthelmintic resistance against Gastrointestinal nematodes of small ruminants.

Anderson, R.C. (2000). Nematode parasites of vertebrates: their development and transmission. *Folia parasitologica, Cabi publishing*., 47-314, 650 pp.

Anene, B. M., Onyekwodiri, E. O., Chime, A. B., & Anika, S. M. (1994). Gastrointestinal parasites in sheep and goats of southeastern Nigeria. *Small Ruminant Research*, *13*(2), 187-192.

Annual Report (2018-19). Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Govt. of India.

Anugrah, S. V., Singh, J. P., Ramakant, N. K. S. & Varun, V. K. Epidemiology of gastrointestinal parasites in goats of Kumarganj region of Uttar Pradesh. Journal of Pharmacognosyand Phytochemistry, pp,16-20.

Armour, J. (1980). The epidemiology of helminth disease in farm animals. *Veterinary Parasitology*, *6*(1-3), 7-46.

Armour, J. (1986). Epidemiology of parasitic gastroenteritis in cattle and sheep. In *Veterinary Record*, Vol. 119, No. 9, pp. 211-211.

Assoku R.K.G. (1981). Studies on the parasitic helminths of sheep and goats in Ghana. Bulletin of Animal Health and Production in Africa, 29, 1-10.

Avares, R. G., Staggemeier, R., Borges, A. L. P., Rodrigues, M. T., Castelan, L. A., Vasconcelos, J., Anschau, M. E., (2011). Spalding SM Molecular techniques for the study and diagnosis of parasite infection . *Journal of Venomous Animals and Toxins including Tropical Diseases*. pages 239-248.

Ayaz, M. M., Raza, M. A., Murtaza, S., & Akhtar, S. (2013). Epidemiological survey of helminths of goats in southern Punjab, Pakistan. *Trop. Biomed*, *30*(1), 62-71.

Azrul, L. M., Poungpong, K., Jittapalapong, S., & Prasanpanich, S. (2017). Descriptive prevalence of gastrointestinal parasites in goats from small farms in Bangkok and vicinity and the associated risk factors. *Annual Research & Review in Biology*, 1-7.

Bandyopadhyay, S., Devi, P., Bera, A., Bandyopadhyay, S., & Bhattacharya, D. (2010). Prevalence of gastrointestinal parasite in goats in Shillong, Meghalaya, India. *Central Parasitology1*(9).

Barger, I. A., Siale, K., Banks, D. J. D., & Le Jambre, L. F. (1994). Rotational grazing for control of gastrointestinal nematodes of goats in a wet tropical environment. *Veterinary Parasitology*, *53*(1-2), 109-116.

Beer, R. J. S. (1973). Morphological descriptions of the egg and larval stages of Trichuris suis Schrank, 1788. *Parasitology*, *67*(3), 263-278.

Bekele, T., Woldeab, T., Lahlou-Kassi, A., & Sherington, J. (1992). Factors affecting morbidity and mortality on-farm and on-station in the Ethiopian highland sheep. *Acta Tropica*, *52*(2-3), 99-109.

Bensch, S., Péarez-Tris, J., Waldenströum, J., & Hellgren, O. (2004). Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation?. *Evolution*, *58*(7), 1617-1621.

Bersissa, K., Tigist, T., Teshale, S., Reta, D., & Bedru, H. (2011). Helminths of sheep and goats in central Oromia (Ethiopia) during the dry season. *Journal of Animal and Veterinary Advances*, *10*(14), 1845-1849.

Bhat, S. A., Mir, M., Allaie, S., Khan, H., Husain, I., & Ali, A. (2011). Comparative resistance of sheep breeds to Haemonchus contortus in pasture infection in Jammu and Kashmir. *J. Vet. Sci. Technol*, *2*, 110.

Biswas, H., Dey, A. R., Begum, N. and Das, P.M. (2014). Epidemiological aspects of gastrointestinal parasites in buffaloes in Bhola, Bangladesh.*Indian J Anim Sci*., 84: 245–250.

Biu, A. A., Maimunatu, A., Salamatu, A. F., & Agbadu, E. T. (2009). A faecal survey of gastrointestinal parasites of ruminants on the University of Maiduguri Research Farm. *Int. J. Biomed. Health Sci*, *5*(4), 175-179.

Blackie, S. (2014). A Review of the Epidemiology of Gastrointestinal Nematode Infections in Sheep and Goats in Ghana. *Journal of Agricultural Science*; Vol. 6, No. 4; 1916-9760.

Blouin, M., & Guay, D. (1997). Activation of ruthenium oxide, iridium oxide, and mixed RuxIr1− x oxide electrodes during cathodic polarization and hydrogen evolution. *Journal of the electrochemical society*, *144*(2), 573.

Bosco, A., Camunas-Soler, J., & Ritort, F. (2014). Elastic properties and secondary structure formation of single-stranded DNA at monovalent and divalent salt conditions. Nucleic acids research, 42(3), 2064-2074.

Bott, N. J., Campbell, B. E., Beveridge, I., Chilton, N. B., Rees, D., Hunt, P. W., & Gasser, R. B. (2009). A combined microscopic-molecular method for the diagnosis of strongylid infections in sheep. *International journal for parasitology*, *39*(11), 1277-1287.

Bowman, D. D. (2002). Georgi's Parasitology for Veterinarians. Saunders Company, 7.

Brahma, A., Pandit, S., Kumar, D., Ghosh, J., & Jas, R. (2015). Prevalence of Gastrointestinal Helminthosis in Black Bengal Goats of Organized farm in West Bengal. *Indian J. Anim. Hlth*, *54*(1), 27-34.

Callejón, R., Nadler, S., De Rojas, M., Zurita, A., Petrášová, J., & Cutillas, C. (2013). Molecular characterization and phylogeny of whipworm nematodes inferred from DNA sequences of cox1 mtDNA and 18S rDNA. *Parasitology research*, *112*(11), 3933-3949.

Campbell R. A., (1971). Biology and Development of *Cotylurus flabelliformis* (Trematoda: Strigeidae).

Chilton, N. B., Gasser, R. B., & Beveridge, I. (1995). Differences in a ribosomal DNA sequence of morphologically indistinguishable species within the Hypodontus macropi complex (Nematoda: Strongyloidea). *International journal for parasitology*, *25*(5), 647-651.

Chowdhury, S. A., Shill, B. K., Hossain, S. M. J. (2007). Chagol palon mannul 2nd Ed. Bangladesh Livestock Research Institute, Savar, Dhaka-1341, January control of gastrointestinal nematodes in goats. *Journal of life and physical sciences*, 3(1): 25-28.

Coleby, P. (1993). Natural Goat Care ("Controlling Goat Parasites- Is It a Losing Battle?").

Colley, D. G., LoVerde, P. T., & Savioli, L. (2001). Medical helminthology in the 21st century.

Connan, R. M. (1976). Effect of lactation on the immune response to gastrointestinal nematodes. The Veterinary *Record*, *99*(24), 476.

Criscione, C. D., Poulin, R., & Blouin, M. S. (2005). Molecular ecology of parasites: elucidating ecological and microevolutionary processes. *Molecular ecology*, *14*(8), 2247-2257.

Crofton, h. D., & Whitlock, j. H. (1965). Ecology and biological plasticity of sheep nematodes. Iv. The biological significance of temperature x time hatching curves for eggs of sheep nematodes. Cornell vet. 55:263-274.

Cutillas, C., Guevara martõÂnez D., Oliveros, R., Guevara, D.C. (1999) Characterization of porcine and ovine Oesophagostomumspp by isoenzymatic patterns and restriction-fragment-length poly-morphisms #RFLPs). *Acta Trop Basel*) 73: 59-71.

Dabasa, G., Shanko, T., Zewdei, W., Jilo, K., Gurmesa, G., & Abdela, N. (2017). Prevalence of small ruminant gastrointestinal parasites infections and associated risk factors in selected districts of Bale zone, south eastern Ethiopia. *Journal of parasitology and vector Biology*, 9(6), 81-88.

Dagnachew, S., Amamute, A., & Temesgen, W. (2011). Epidemiology of gastrointestinal helminthiasis of small ruminants in selected sites of North Gondar zone, Northwest Ethiopia. *Ethiopian Veterinary Journal*, *15*(2).

Dahlgren, S. S. and Gjerde, B. (2010). The red fox (Vulpesvulpes) and the arctic fox (Vulpeslagopus) are definitive hosts of Sarcocystisalces and Sarcocystishjorti from moose (Alcesalces). *Parasitology* 137, 1547-1557.

Dallas, J. F., Irvine, R. J., Halvorsen, O., & Albon, S. D. (2000). Identification by polymerase chain reaction (PCR) of Marshallagia marshalli and Ostertagia gruehneri from Svalbard reindeer. *International Journal for Parasitology*, *30*(7), 863-866.

Dangoudoubiyam, S., Vemulapalli R., and Kazacos, K. R., (2009). PCR Assays for Detection of Baylisascarisprocyonis Eggs and Larvae. *Parasitol. J.*, 95(3) 571-577.

Das, M., Laha, R., Goswami, A., & Goswami, A. (2017). Gastrointestinal parasitism of goats in hilly region of Meghalaya, India. *Veterinary world*, *10*(1), 81.

Demelash B., Yilma J. and Hassen C. (2006). Ovine helminthosis is major health constraints to productivity of sheep in Ethiopia, Faculty of Veterinary Medicine, Awassa University, Awassa, Ethiopia. pp. 65-90.

Demelash, B., Inangolet, F., Oloya, J., Asseged, B., Badaso, M., Yilkal, A., Skjerve, E. Prevalence of bovine tuberculosis in Ethiopian slaughter cattle based on post-mortem examination. *Tropical Animal Health and Production*. 41, 755–765.

Demelash, K., Abebaw, M., Negash, A., Alene, B., Zemene, M., & Tilahun, M. (2016). A Review on Diagnostic Techniques in Veterinary Helminthlogy. *Nature and Science, 14*(7), 109-118.

Demissie, T., Tesfaye, D., Fekadu, A., & Asefa, I. (2013). Study on abomasal nematodes of sheep and goats: Comparison and characterization of vulvar morphology of Haemonchus in Hawassa, Ethiopia. *African Journal of Agricultural Research*, *8*(41), 5181-5186.

Dereje, S. (2008). Study on prevalence of major GI nematodiasis of small ruminants in three selected sites of Afar Region, Ethiopia. *Master of Science thesis in*

*Tropical Veterinary Parasitology. School of Graduate Studies, Addis Ababa University*, 72.

Dhand, Navneet K., Evan Sergeant, Jenny-Ann LML Toribio, & Richard J. Whittington. "Estimation of sensitivity and flock-sensitivity of pooled faecal culture for Mycobacterium avium subsp. paratuberculosis in sheep." Preventive veterinary medicine 95, no. 3-4: 248-257.

Dhar, D. N., Sharma, R. L., & Bansal, G. C. (1982). Gastro-intestinal nematodes in sheep in Kashmir. *Veterinary Parasitology*, 11(2-3), 271-277.

Dinani, O. P., Tyagi, P. K., Giri, A. K., & Popat, D. S. (2018). Role of livestock in doubling the farmers' income-national perspective and the way forward. *International Journal of Science, Environment and Technology*, *7*(2), 496-504.

Donald, A. D. (1968). Ecology of the free-living stages of nematode parasites of sheep. Australian Veterinary Journal, 44(4).

Dorris, M., Viney, M.E & Blaxter, M.L. (2002). Molecular phylogenetic analysis of the genus Strongyloide sandrelated nematodes. *International Journal for Parasitology* 32, 1507 – 1517.

Dorris, M., De Ley, P. & Blaxter, M. L. (1999) Molecular analysis of nematode diversity and the evolution of parasitism. *ParasitolToday* 15: 188-193.

Edosomwan, E. U. & Ewarami T. J. (2012). Ticks and helminth parasites of Cattle atIkpoba hill abattoir Benin – City, Edo.

El-Azazy O. (1990). Absence of hypobiosis inabomasal nematodes of sheep and goats in Egypt.Veterinary Parasitology 37: 55-60.

El-Azazy, O. M. E. (1995). Seasonal changes and inhibited development of the abomasal nematodes of sheep and goats in Saudi Arabia. *Veterinary Parasitology*, *58*(1-2), 91-98.

Emiru, B., Amede, Y., Tigre, W., Feyera, T., & Deressa, B. (2013). Epidemiology of gastrointestinal parasites of small ruminants in Gechi District, Southwest Ethiopia. *Advances in Biological Research*, *7*(5), 169-174.

Eslami, A. H., & Nabavi, L. (1976). Species of gastro-intestinal nematodes of sheep from Iran. *Bulletin de la Société de Pathologie Exotique*, *69*(1), 92-95.

Faizal, A. C. M., Rajapakse, R. P. V. J., Jayasinghe, S. R., & Rupasinghe, V. (1999). Prevalence of Eimeria spp. and gastrointestinal nematodes versus weight gains in treated goats raised in the dry areas of Sri Lanka. *Small Ruminant Research*, *34*(1), 21-25.

Fakae, B. B., & Chiejina, S. N. (1993). The prevalence of concurrent trypanosome and gastrointestinal nematode infections in West African Dwarf sheep and goats in Nsukka area of eastern Nigeria. *Veterinary Parasitology*, *49*(2-4), 313-318.

FAO (1992). Distribution and impact of helminth diseases of livestock in developing countries. FAO Animal Production and Health paper, 96..

FAO (2008). Distribution and impact of helminth diseases of livestock in developing countries. FAO Animal Production and Health Paper. Vol. 96. FAO, Rome, 221.

Fatima, M., Saeed, S., Shaikh, R. S., Ali, M., & Iqbal, F. (2015). A study on molecular detection of Theileria lestoquardi by PCR amplification in apparently healthy small ruminants from five districts of Southern Punjab. Pakistan Journal of Zoology, 47(2).

Felsenstein, J. and Felenstein, J. (2004). *Inferring phylogenies* (Vol. 2, p. 664). Sunderland, MA: Sinauer associates.

Fivaz, B. H., Horak, I. G., & Williams, E. J. (1990). Helminth and arthropod parasites of Angora goats on irrigated Kikuyu grass pastures in the eastern Cape Province. *Journal of the South African Veterinary Association*, *61*(3), 112-116.

Food and Agriculture Organization (FAO) (2002) Biological control of nematode parasites of small ruminants in Asia. FAO Animal Prod. Health Paper 20, 1-100.

Food and Agriculture Organization (FAO), (2010). Breeding strategies for sustainable management of animal genetic resources. Animal Production and Health

Guidelines, No.3. Food and Agriculture Organization of the United Nations, Rome, Italy.

Gadahi, J. A., Arshed, M. J., Ali, Q., Javid, S. B. & Shah, S. I. (2009). Prevalence of gastrointestinal parasites of sheep and goats in and around Rawalpinidi. PakistanVeterinary World. 2:52-53.

Gaherwal, S., Prakash, M. M., & Dudwe, J. (2016). Prevalence and incidence of nematodes in goats at five different villages of Barwani district (MP). Int J Adv Res, 4, 1126-1137.

Gall, C. (1981). Goat Production. Academic Press, London/New York.

Gamit, A., (2018). A slaughter house report of oesophagostomosis in goat. *International Journal of science, Environment* vol. 7, no 1, 116 – 120.

Garedaghi, Y., Rezaii-Saber, A. P., Naghizadeh, A. & Nazeri, M. (2011) Survey on prevalence of sheep and goats lungworms in Tabriz abattoir, Iran.Adv. Environ. Bio. 5:773-775.

Gasser R.B., De Gruijter J.M. and Polderman A.M. (2006). Insights into the epidemiology and genetic make-up of Oesophagostomum bifurcum from human and non-human primates using moleculartools. *Parasitology,* 132, 453–460.

Gasser, R. B., & Newton, S. E. (2000). Genomic and genetic research on bursate nematodes: significance, implications and prospects. *International journal for parasitology*, *30*(4), 509-534.

Gasser, R. B., Chilton, N. B., Hoste, H., & Beveridge, I. (1993). Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic acids research*, *21*(10), 2525.

Gatongi, P. M. (1996). Epidemiology and control of haemonchosis of small ruminants in semi-arid Kenya. Kenya Agricultural Research Institute Information Bulletin, (17), 1-334.

Gebeyehu, E. B., Seo, M. G., Jung, B. Y., Byun, J. W., Oem, J. G., Kim, H. Y., & Kwak, D. (2013). Prevalence of gastrointestinal parasites in Korean native goats (Capra hircus aegagrus). *Journal of Animal and Plant Sciences*, *23*(4), 986-989.

Getachew, T., Urgessa, F. & Yacob, H. (2013). Field investigation of anthelmintic efficacy and risk factors for anthelmintics drug resistance in sheep at Bedelle District of Oromia Region, Ethiopia. Ethiop. Vet. J17(2): 37-49.

Ghai, R. R, Chapman, C. A., Omeja, P. A., Davis, T. J. and Goldberg, T. L. (2014). Nodule worm infection in humans and wild primates in Uganda: cryptic species in a newly identified region of human transmission. *Trop. Dis*.8, 26-41.

Gharamah, A. A., Azizah, M. S., & Rahman, W. A. (2012). Genetic variation of Haemonchus contortus (Trichostrongylidae) in sheep and goats from Malaysia and Yemen. *Veterinary parasitology*, *188*(3-4), 268-276.

Ghasemikhah, R., Tabatabaiefar, M. A., Shariatzadeh, S. A., Shahbazi, A., & Hazratian, T. (2017). A PCR-based molecular detection of Strongyloides stercoralis in human stool samples from Tabriz City, Iran. *Scientia pharmaceutica*, *85*(2), 17.

Gilbert A. C., (1996). Chapter 86 Helminths: Structure, Classification, Growth, and Development, NCBI Bookshelf. A service of the National Library of Medicine, National Institutes of Health. Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston.

Githigia, S. M., Thamsborg, S. M., Munyua, W. K., & Maingi, N. (2001). Impacof gastrointestinal helminths on production in goats in Kenya. *Small Ruminant Research*, *42*(1), 21-29.

GOI (2016). Agricultural Statistics at a Glance, Directorate of Economics and Statistics, Ministry of Agriculture, Government of India, New Delhi.

Goldberg A. (1952) Journal of Parasitology, 38(35), 35 -47.

Goodey, T. (1924). The anatomy of *Oesophagostomum dentatum* (Rud.) a nematode parasite of the pig, with observations on the structure and biology of the free-living larvae. *Journal of Helminthology*, 2, 1-14.

Grant, J. L. (1981). The epizootiology of nematode parasites of sheep in a high-rainfall area of Zimbabwe.

Hagardson, K. (2006). Comparison of DNA isolation methods to detect Leishmania parasites in blood samples.

Hall, B. G. (2011). Phylogenetic trees made easy: A how to manual (No. 576.88 H174p) Sinauer.

Hansen, J. & Perry, B., 1994. The Epidemiology,

Harris, T. H., Szalanski, A. L., & Powers, T. O. (1999). *Molecular Identification of Nematodes Manual*. University of Nebraska.

Hasheminasab, S. S., Jalousian, F., & Meshgi, B. (2016). Molecular and morphological characterization of Parabronema skrjabini of sheep and goats at three different geographical zones in Iran. *Annals of Parasitology*, *62*(1).

Hassan, M. M., Hoque, M. A., Islam, S. K. M. A., Khan, S. A., Roy, K., & Banu, Q. (2011). A prevalence of parasites in black bengal goats in Chittagong, Bangladesh. *International Journal of Livestock Production*, *2*(4), 40-44.

Hassan, S. A., Mohammed, A. A., & Omer, F. A. (2017). Molecular method to diagnosis of some strongylide nematode of goats in Nyala area South Darfur State-Sudan. *Journal of Agriculture and Veterinary Science*, *10*(4), 54-56.

Hassan, S. A., Mohammed, A. A., & Omer, F. A. (2017). Molecular method to diagnosis of some strongylide nematode of goats in Nyala area South Darfur State-Sudan. *Journal of Agriculture and Veterinary Science*, *10*(4), 54-56.

Hawkins, P.A., 1(945). Studies of sheep parasites VI. observations on weather in relation to untreated nematode infections. *J. Parasitol.* 31:17.

Heise, M., Epe, C., & Schnieder, T. (1999). Differences in the second internal transcribed spacer (ITS-2) of eight species of gastrointestinal nematodes of ruminants. *The Journal of parasitology*, 431-435.

Herlich, H. (1978). The importance of helminth infections in ruminants. *Revue Mondiale de Zootechnie (FAO)-Revista Mundial de Zootecnia (FAO)*.

Holmes, P. H. (1985). Pathogenesis of trichostrongylosis. *Veterinary parasitology*, *18*(2), 89-101.

Holmes, P. H. (1993). Interactions between parasites and animal nutrition: the veterinary consequences. The Proceedings of the Nutrition Society, 52 (1), 113-120.

Hoste, H, Chartier, C. & Le Frileux, Y. (2002). Control of gastrointestinal parasitism with nematodes in dairy goats by treating the host category at risk. *Veterinary Research*, 33(5), 5 3 1-5 4 5.

Hoste, H., Torres-Acosta, J. F. J., & Aguilar- Caballero, A. J. (2008). Nutrition–parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes?. *Parasite immunology*, *30*(2), 79-88.

https://en.wikipedia.org/wiki/Haemonchus_contortus

Hussain, Q. (1985). Studies on the incidence of gastro-intestinal parasites and efficacy of Banminth-II against nematodes in buffalo calves.

IEMVT (1980). Les petits ruminants d'Afrique de l'Ouest. Synthése des connaissances actuelles.

Indre, D., Dărăbuş, G. H., Oprescu, I., Morariu, S., Mederle, N. A. R. C. I. S. A., Ilie, M. S., ... & Imre, M. (2010). Morphometrical studies on some eggs of gastrointestinal nematodes from sheep. *Lucrări Ştiinłifice Medicină Veterinară*, *1*, 30-35.

Islam, M. S., Shahadat, M, H.., Dey A. R., AliM., M. A., Akter S & Alam, M. Z., 2017., Epidemiology of gastrointestinal parasites of small ruminants in Mymensingh, Bangladesh. *Journal of Advanced Veterinary and Animal Research* 2311-7710.

Jacquiet, P., Humbert, J. F., Comes, A. M., Cabaret, J., Thiam, A., & Cheikh, D. (1995). Ecological, morphological and genetic characterization of sympatric Haemonchus spp. parasites of domestic ruminants in Mauritania. *Parasitology*, *110*(4), 483-492.

Jan, A., Shah, H., Ahmad, I., Younas, M., & Haroon, R. U. (2015). Prevalence and Comparison of ovine gastrointestinal helminthes parasites in domesticated and farmed, male and female sheep at University Town Peshawar, Pakistan. *Pakistan. Journal of Entomology and Zoology Studies*, *3*, 350-353.

Jas, R., & Ghosh, J. D. (2009). Economic impact of gastrointestinal nematodosis in sheep: enhanced meat production by anthelmintic treatment. *Indian Journal of Animal Sciences*, *79*(8), 761-763.

Jasmer, D. P., Wescott, R. B., & Crane, J. W. (1987). Survival of third-stage larvae of Washington isolates of Haemonchus contortus and Ostertagia circumcincta exposed to cold temperatures. *Proceedings of the Helminthological Society of Washington*, *54*(1), 48-52.

Jegede, O. C., Adejoh, A. A., Obeta, S. S., & Olayemi, O. D. (2015). Gastrointestinal Parasites of Sheep and Goats in Gwagwalada Area Council, Federal Capital Territory, Abuja, Nigeria; with a Special Reference to sex, breed and age. *Alexandria Journal for Veterinary Sciences*, *46*(1).

Jegede, O. C., Rabiu, B. M., Obeta, S. S., Malang, S. K., & Ejiofor, C. E. (2013). Gastrointestinal parasites of ruminants slaughtered in Gwagwalada Abattoir, Federal Capital Territory, Abuja, Nigeria. *Nigerian Jouirnal of Parsitology*, *34*(1), 55-59.

Jithendran, K. P. (2000). Helminth Parasites-A Constraint in animal health management in Himachal Pradesh.

Jithendran, K.P. & Bhat, T.K. (1999). Epidemiology of parasites in dairy animals in the north-west humid Himalayan region of India with particular reference Himalayan region of India with particular reference to gastro-intestinal nematodes. *Trop. Anim. Health Prod*., 31: 205-214.

Junaidu, A. U. & Adamu, S. G., (1997) "Survey of Gastrointestinal helminth parasites of dogs of public health importance in Sokoto Metropolis," in Proceedings of the 22nd Annlual Conference of the Nigerian society for Animal Production, Abubaka Tafawa Balewa University Press.

Jurasek, M.E., Bishop-Stewart, J.K., Storey, B.E., Kaplan, R.M. & Kent, M.L., 2010. Modification and further evaluation of a flourescein-labeled peanut agglutinin test for identification of *Haemonchus contortus* eggs, *Vet. Parasitol*, 169, 209-213.

Justine, J. L., Briand, M. J., & Bray, R. A. (2012). A quick and simple method, usable in the field, for collecting parasites in suitable condition for both morphological and molecular studies. *Parasitology Research*, *111*(1), 341-351.

Kabir, M. H. B., Mondal, M. M. H., Eliyas, M., Mannan, M. A., Hashem, M. A., Debnath, N. C., ... & Elahi, M. F. (2011). An epidemiological survey on investigation of tick infestation in cattle at Chittagong District, Bangladesh. *African Journal of Microbiology Research*, *5*(4), 346-352.

Karp, A., Seberg, O. L. E., & Buiatti, M. (1996). Molecular techniques in the assessment of botanical diversity. *Annals of Botany*, *78*(2), 143-149.

Katoch, R., Mandial, R. K., & Nagal, K. B. (1999). Outbreak of *Haemonchus contortus* infection in sheep of Himachal Pradesh. *Indian veterinary journal*, *76*(10), 932-933.

KedarKarki, Bashir, B. K., and Subedi, J. R., (2012). A case Study on Seasonal Prevalence of Helminth Parasites inGoats (Capra Hircus) in Kalanki (Khasibazzar), Kathmandu Nepal Pharmacology & Life Sciences, *Journal's URL*. 11-13 ISSN 2277-1808.

Keyyu, J. D., Kassuku, A. A., Kyvsgaard, N. C., & Willingham, A. L. (2003). Gastrointestinal nematodes in indigenous Zebu cattle under pastoral and nomadic management systems in the lower plain of the Southern Highlands of Tanzania. *Veterinary Research Communications*, *27*(5), 371-380.

Khan, A. I., and Kishor, P. (2014). Haemato-biochemical changes in caprine helminthosis treated with polyherbal anthelmintics Livestock Research International. P-59-62.

Khan, M.N., Sajid, M.S., Khan, M.K., Iqbal, Z. & Hussain, A. (2010) Gastrointestinal helminthiasis: prevalence and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan. *Parasitol Res.;* 107(4):787–794.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, *16*(2), 111-120.

Krecek, R. C., Boomker, J., Penzhorn, B. L., & Scheepers, L. (1990). Internal parasites of giraffes (Giraffa camelopardalis angolensis) from Etosha National Park, Namibia. *Journal of Wildlife Diseases*, *26*(3), 395-397.

Kuchai, J. A., Ahmad, F., Chishti, M. Z., Tak, H., Javid, A., Ahmad, S., & Rasool, M. (2012). A study on morphology and morphometry of *Haemonchus contortus*. *Pakistan Journal of Zoology*, *44*(6).

Kumar, S., Jakhar, K. K., & Singh, A. D. (2015). Etio-pathological investigations to study the gross and histopathological lesions affecting gastrointestinal tract of sheep. *Scientific Research and Essays*, *10*(10), 356-361.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, *33*(7), 1870-1874.

Kumba, F. F., Katjivena, H., Kauta, G., & Lutaaya, E. (2003). Seasonal evolution of faecal egg output by gastrointestinal worms in goats on communal farms in

eastern Namibia. *Onderstepoort Journal of Veterinary Research*, *70*(4), 265-271.

Kumsa, B., & Wossene, A. (2007). Abomasal nematodes of small ruminants of Ogaden region, eastern Ethiopia: prevalence, worm burden and species composition. *Revue de médecine vétérinaire*, *158*(1), 27.

Kumsa, B., Tigist, T., Teshale, R. & Bedru, H. (2011). Helminthes of sheep and goats in central Ethiopia during the Dry season. *J. Anim. And Vet. Advances*10 (4): 1845-1849.

Laksmi, H. D., Jagannath M S and Souza, P. E. D., (2001). Gastrointestinal parasitic infections in sheep at different forms of Karnataka. *J. Veterinary Parasitology*, 15: 133-135.

LeJambre, L. F., Ractliffe, L. H., Whitlock, J. H., & Crofton, H. D. (1970). Polymorphism and egg-size in the sheep nematode, Haemonchus contortus. *Evolution*, 625-631.

Levine, N. D. 1963. Weather, climate and the bionomics of ruminant nematode larvae. Adv. *Vet. Sci*. 8:215-261.

Lichtenfels, J. R., Pilitt, P. A., & Hoberg, E. P. (1994). New morphological characters for identifying individual specimens of Haemonchus spp.(Nematoda: Trichostrongyloidea) and a key to species in ruminants of North America. *The Journal of parasitology*, 107-119.

Lichtenfels, J. R., Pilitt, P. A., & Le Jambre, L. F. (1986). Cuticular ridge patterns of Haemonchus contortus and Haemonchus placei (Nematoda: Trichostrongyloidea). *Proceedings of the Helminthological Society of Washington*, *53*(1), 94-101.

Lin, Q., Li, H. M., Gao, M., Wang, X.Y., Ren, W.X., Cong, M.M., Tan, X.C., Chen, C.X., Yu, S.K. & Zhao, G.H. (2012). Characterization of Baylisascaris schroederi from Qinling subspecies of giant panda in China by the first internal

transcribed spacer (ITS-1) of nuclear ribosomal DNA. *Parasitol Res*. 110, 1297-1303.

Linstow, O. V. (1906). Helminthes from the collection of the Colombo Museum. *Spolia Zeylanica*, *3*(2), 163-188.

Littlewood, D. T. J., & Bray, R. A. (Eds.). (2000). *Interrelationships of the Platyhelminthes*. CRC Press.

Liu, G. H., Zhou, W., Nisbet, A. J., Xu, M. J., Zhou, D. H., Zhao, G. H., Wang, S. K., Song H. Q., Lin R. Q., Zhu, X. Q., (2014). Characterization of Trichuris trichiurafrom humans and T. suisfrom pigs in China using internal transcribed spacers of nuclear ribosomal DNA.J Helminthol. 88: 64-68.

Lloyd, S. (1983). Effect of pregnancy and lactation upon infection. *Veterinary Immunology and Immunopathology*, *4*(1-2), 153-176.

Lone, B. A., Chishti, M. Z., Ahmad, F., & Tak, H. (2012). A survey of gastrointestinal helminth parasites of slaughtered sheep and goats in Ganderbal, Kashmir. *Global Veterinaria*, *8*(4), 338-341.

Luton, K., Walker, D., & Blair, D. (1992). Comparisons of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). *Molecular and Biochemical Parasitology*, *56*(2), 323-327.

Mabe, L. T. (2012). Development of molecular diagnostic methods (LAMP and PCR) for detection of Haemonchus contortus, Fasciola SPP and Trichostrongylus SPP infections in livestock (Doctoral dissertation, University of the Free State).

Maingi, N. (1995). Epidemiology and Control of Gastrointestinal Nematodes of Ruminants in Kegna. 6th Symposium on Helminth and Control. October 6, 1995. Faculty of Veterinary Medicine, Utrechet Univeristy, the Netherlands.pp 49-51.

Maingi, N., Gichigi, M. N., & Njoroge, G. K. (2001). Gastrointestinal nematode infections in sheep grazing on communal land in Nyandarua District of Central

Kenya in relation to deworming practices. *Bull Anim Health Prod (Africa)*, *49*, 153-161.

Maiti, S. K., Rao, V. N., Pal, S., & Ali, S. L. (1999). Clinico-haematological and therapeutic studies in parasitic gastroenteritis in sheep. *Indian veterinary journal*, *76*(5), 435-437.

Mandal, A., Karunakaran, M., Rout, P. K., & Roy, R. (2014). Conservation of threatened goat breeds in India. *Animal Genetic Resources/Resources génétiques animales/Recursos genéticos animales*, *55*, 47-55.

Mattos-Graner, R. D. O., Zelante, F., Line, R. C. S. R., & Mayer, M. P. A. (1998). Association between caries prevalence and clinical, microbiological and dietary variables in 1.0 to 2.5-year-old Brazilian children. *Caries research*, 32(5), 319-323.

Mbaria, J. M., McDermott, J. J., Kyule, M. N., & Gichanga, E. J. (1995). Prevalence of helminths and helminth control practices in Molo Division, Nakuru district, Kenya.

Mbuh, J. V., Ndamukong, K. J. N., Ntonifor, N., & Nforlem, G. F. (2008). Parasites of sheep and goats and their prevalence in Bokova, a rural area of Buea Sub Division, Cameroon. *Veterinary parasitology*, *156*(3-4), 350-352.

McKenna, P. B. (2010). Update on the prevalence of anthelmintic resistance in gastrointestinal nematodes of sheep in New Zealand. *New Zealand veterinary journal*, *58*(3), 172-173.

*Medterms.com. (2000). Trichuris trichiura* definition - Medical Dictionary definitions of popular medical terms easily defined on MedTerms*". 04-15. Retrieved 2009-05-19.*

Mehmood, K., Ijaz, M., Khan, M. S., Khan, M. A., Sabir, A. J., & Rehman, A. (2012, April). Prevalence of Gastrointestinal Parasites and Efficacy of Anthelmintics in Dairy Goats in Pakistan. In *first asia dairy goat conference* (vol. 9, p. 165).

Meshgi, B., Jalousian, F., & Masih, Z. (2015). Phylogenetic Study of Haemonchus Species from Iran Based On Morpho-Molecular Characterization. *Iranian journal of parasitology*, *10*(2), 189.

Minami, T., Kami, D. T., Nogami, S., Saitoh, and Schimura, K. (2001). Technical manual for the examination and control of parasites of domestic animals. *Japan Livestock Technology Association*. Pp. 1-10.

Misal, G., Borde, S., Thosar, A., & Lohat, S. (2015). Morphological and Molecular Analysis of Trichuris Species (Nematoda: Trichuridae) From Capra Hircus (L.) in Aurangabad District (MS) India. *International Journal of Scientific Reseach. Vol*, (4), 8.

Mohammed, A., Haimanot, D., Tadele, K., Tilahun, Z. and Girma, K. (2015). Gastrointestinal nematodes of sheep in GursumWoreda of Eastern Hararghe Zone, Oromia Regional State, Ethiopia. *Researcher* 7(8): 45-54.

Mohanta, U. K., Anisuzzaman, A., Farjana, T., Das, P. M., Majumder, S., & Mondal, M. M. H. (2007). Prevalence, population dynamics and pathological effects of intestinal helminths in Black Bengal goats. *Bangladesh Journal of Veterinary Medicine*, 63-69.

Mohanta, U. K., Anisuzzaman, Farjana T., Das, P. M., Majumder, S., Mondal, M. M. H. (2007). Prevalence, population dynamics. *J Vet Med*, 563-569.

Morgan, E. R., Torgerson, P. R., Shaikenov, B. S., Usenbayev, A. E., Moore, A. B. M., Medley, G. F., & Milner-Gulland, E. J. (2006). Agricultural restructuring and gastrointestinal parasitism in domestic ruminants on the rangelands of Kazakhstan. *Veterinary Parasitology*, *139*(1-3), 180-191.

Mulugeta, T., Geremew, B., & Molalegne, B. (2010). Prevalence of gastrointestinal parasites of sheep and goats in and around Bedelle, South-Western Ethiopia. *Internet Journal of Veterinary Medicine*, *8*(2).

Muluneh, J., Bogale, B., & Chanie, M. (2012). Major gastrointestinal nematodes of small ruminants in Dembia District, Northwest Ethiopia. *Europ. J. Appl. Sci.*, 6, 30-36.

Nabi, H., Saeed, K., Shah, S. R., Rashid, M. I., Akbar, H. & Shehzad, W. (2014). Epidemiological study of gastrointestinal nematodes of goats in district Swat, Khyber Pakhtunkhwa, Pakistan. *Sci. Int*.26(1), 283–286.

Nakanishi, Y., Takayama, K. & Yasuda, N. (2011). Effects of Japanese Bead-tree (Melia azedarach var. subtripinnata) on Gastrointestinal Parasites in Goats. JARQ 45(1), 117- 121.

Naomi J. F., Glenn M., Ross, S., Davidson P. C. L., White, & Michael R. H. (2012). Livestock Helminths in a Changing Climate: Approaches and Restrictions to Meaningful Predictions Animals, 2, 93-107; 2076-2615.

Nei, M. & Kumar, S. (2000). Molecular evolution and phylogenetics, New York Oxford University Press.

Nizam, M. (2013). Haematological and biochemical studies of helminth infected goats in South Kashmir (Doctoral dissertation, M. Sc. thesis, University of Kashmir).

Njau, B. C., Scholtens, R. G., & Kasali, O. (1990). Parasites of sheep at the international livestock centre for Africa debre berhan station, Ethiopia. *Preventive Veterinary Medicine*, *9*(4), 267-277.

Nsereko, G., Emudong, P., Mulindwa, H., & Okwee-Acai, J. (2015). Prevalence of common gastro-intestinal nematode infections in commercial goat farms in Central Uganda. *Uganda Journal of Agricultural Sciences*, *16*(1), 99-106.

Nwosu, C. O., Madu, P. P., & Richards, W. S. (2007). Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of north-eastern Nigeria. *Veterinary parasitology*, *144*(1-2), 118-124.

Nwosu, C. O., Ogunrinade, A. F., & Fagbemi, B. O. (1996). Prevalence and seasonal changes in the gastrointestinal helminths of Nigerian goats. *Journal of Helminthology*, *70*(4), 329-334.

O'Connor, L. J., Walkden-Brown, S. W., & Kahn, L. P. (2006). Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Veterinary parasitology*, *142*(1-2), 1-15.

Odoi A, Gathuma J.M., Gachuiri, C.K. & Omore, A. (2007). Risk factors of gastrointestinal nematode parasite infections in small ruminants kept in smallholder mixed farms in Kenya. *BMC Veterinary Research*, 3, 1746-6148.

Oldham, P., Hall, S., Barnes, C., Hoffman, I., & Boettcher, P. (2014). Patent Landscape Report on Animal Genetic Resources. *World Intellectual Property Organization (WIPO), Geneva, Switzerland*.

Opara, M. N., Nwaobasi, J. K., & Okoli, I. C. (2005). Occurrence of parasitic helminths among small ruminants reared under traditional husbandry system in Owerri, South East Nigeria Presence des helminthes chez les petits ruminants en elevage traditionnel a Owerri dans le Sud-est du Nigeria. *Bulletin of Animal Health and Production in Africa*, *53*(4), 226-233.

Osakwe, I. I., & Anyigor, S. I. (2007). Prevalence of gastrointestinal helminths in West African Dwarf (WAD) goats in an agrarian agro-ecosystem. *Animal Research International*, *4*(3).

Ota N., Hasegawa, H., McLennan, M. R., Kooriyama, T., Sato, H., Pebsworth, P. A., Huffman, M. A. (2015). Molecular identification of Oesophagostomum spp. from 'village' chimpanzees in Uganda and their phylogenetic relationship with those of other primates. *R. Soc. open sci.*2, 150-471.

Owhoeli, O., Elele, K., & Gboeloh, L. B. (2014). Prevalence of gastrointestinal helminths in exotic and indigenous goats slaughtered in selected abattoirs in Port Harcourt, south-south, Nigeria. *Chinese Journal of Biology, 2014*.

Pal, R. A. & Qayyum, M. (1993). "Prevalence of gastrointestinal nematodes of sheep and goats in upper Punjab," *Pakistan Veterinary Journal*, vol. 13, pp. 138–141.

Pal, R. A., & Qayyum, M. A. Z. H. A. R. (1992). Breed, age and sex-wise distribution of gastro-intestinal helminths of sheep and goats in and around Rawalpindi region. *Pak. Vet. J*, *12*(2), 60-63.

Panesar, T. S., & Croll, N. A. (1980). The location of parasites within their hosts: site selection by Trichuris muris in the laboratory mouse. *Inter J Parasitology*, 10, 261-273.

Patchamuthu, R. (1993). Teknik-teknikasas alid pemasangan seluruh (wholemount) dan teknik-teknik mikroskopdan penjagaannya. PPSKH, USM. 33pp.

Pathak, A. K., and Pal, S. (2008). Seasonal prevalence of gastrointestinal parasites in goats from Durg district of Chhattisgarh. Veterinary World 1(5): 136-137.

Patnode, M. L., Bando, J. K., Krummel, M. F., Locksley, R. M., & Rosen, S. D. (2014). Leukotriene B4 amplifies eosinophil accumulation in response to nematodes. *J Exp Med*, 211, 1281-1288.

Pedreira, J., Paz-Silva, A., Sánchez-Andrade, R., Suarez, J. L., Arias, M., Lomba, C., ... & Morrondo, P. (2006). Prevalences of gastrointestinal parasites in sheep and parasite-control practices in NW Spain. *Preventive veterinary medicine*, *75*(1-2), 56-62.

Piarroux, R., Gambarelli, F., Dumon, H., Fontes, M., Dunan, S., Mary, C., ... & Quilici, M. (1994). Comparison of PCR with direct examination of bone marrow aspiration, myeloculture, and serology for diagnosis of visceral Leishmaniasis in immunocompromised patients. *Journal of clinical microbiology*, *32*(3), 746-749.

Poddar, P. R., Begum, N., Alim, M. A., Dey, A. R., Hossain, M. S., Labony, S. S., Prevalence of gastrointestinal helminths of sheep in Sherpur, Bangladesh. *Journal of Advanced Veterinary and Animal Research.* 4, 274–280.

Pouillevet, H., Dibakou, S. E., Ngoubangoye, B., Poirotte, C., & Charpentier, M. J. (2017). A comparative study of four methods for the detection of nematode eggs and large protozoan cysts in mandrill faecal material. *Folia Primatologica*, *88* (4), 344-357.

Preston J.M. and Allonby E.W. (1979). The influence of breed on the susceptibility of sheep of Haemonchus contortus infection in Kenya. *Research in Veterinary Science* 26, 134-139.

Probert, A. J., 1994. Chemotherapy of helminthes of livestock and man. In: Chowdhury, N. and Tada, I. (Eds.). *Helminthology*. *Verlag*, pp 315-334.

Punja, Z., De Boer, S. H., and Sanfacon H., (2008). Biotechnology and plant disease management.pp.195.

Qayyum, M. (1993). Prevalence of gastrointestinal nematodes of sheep and goats in upper Punjab, Pakistan. *Pakistan Veterinary Journal*, *13*, 138-138.

Qazi, F., Khalid, A., Poddar, A., Tetienne, J. P., Nadarajah, A., Aburto-Medina, A., & Tomljenovic-Hanic, S. (2020). Real-time detection and identification of nematode eggs genus and species through optical imaging. *Scientific Reports*, *10*(1), 1-12.

Radhakrishnan, L., Gomathinayagam, S., & Balakrishnan, V. (2010). Evaluation of anthelmintic effect of neem (Azadirachta indica) leaves on Haemonchus contortus in goats. *Research Journal of Parasitology*, *5*(4), 292-297.

Radiostits, O. M., Gray, C. C., Hinchelift, K. W. & Constable, P. D., (2007). Veterinary Medicine. Atextbook of the disease of cattle, horses, sheep, pigs and goats,10thedition, Sunders, Saunders Elsevier, London.

Radostits, O. M., Blood, D. C., & Gay, C. C. (1994). Diseases caused by helminth parasites. *Veterinary Medicine: a textbook of diseases of cattle, sheep, pigs, goats and horses, 8th Edition. London, Balliere Tindall,* 1223-1230.

Rahman, W. A., & Collins, G. H. (1992). An association of faecal egg counts and prolactin concentrations in sera of periparturient Angora goats. *Veterinary Parasitology*, *43*(1-2), 85-91.

Rahman, W. A., & Hamid, S. A. (2007). Morphological characterization of Haemonchus contortus in goats (Capra hircus) and sheep (Ovis aries) in Penang, Malaysia. *Tropical biomedicine*, *24*(1), 23-27.

Raza, M. A., Iqbal, Z., Jabbar, A., & Yaseen, M. (2007). Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. *Journal of Helminthology*, *81*(3), 323.

Raza, M. A., Younas, M., & Schlecht, E. (2014). Prevalence of gastrointestinal helminths in pastoral sheep and goat flocks in the Cholistan desert of Pakistan. *The Journal of Animal & Plant Sciences*, *24*(1), 127-134.

Regassa, F., Teshale, S., Reta, D. & Yosef, K. (2007) Epidemiology of gastrointestinal parasites of ruminants in Western Oromia, Ethiopia. *Int. J. Appl. Res. Vet*. Med. 4, 51-57.

Rey, B. (1991). Small ruminant genetic resources and parasite challenge in sub-Sahara, Africa. In: Proceedings of the Research Planning Workshop Held at ILCA on Resistance to Endoparasites in Small Ruminants, Addis Ababa, Ethiopia, p. 23-32.

Reyaz, A. (2005). Epidemiology, Biology, and Pathogenicity of Nematodes in Ruminants of Kashmir. M. *Phil. Dissertations. University of Kashmir*.

Roberts, M. G., & Grenfell, B. T. (1992). The population dynamics of nematode infections of ruminants: The effect of seasonally in the free-living stages. *Mathematical Medicine and Biology: A Journal of the IMA*, *9*(1), 29-41.

Rosenthal, B. M., Dunams, D. B., & Pritt, B. (2008). Restricted genetic diversity in the ubiquitous cattle parasite, Sarcocystis cruzi. *Infection, Genetics and Evolution*, *8*(5), 588-592.

Sacher, G. (1966). The complementarity between development and aging-experimental and theoretical considerations. In New York Acad. *Sci. Conf. on Interdisciplinary Perspectives of Time*.

Sahai, B. N., & Deo, P. G. (1964). Studies on Haemonchus contortus (Rudolphi, 1803) Cobbold (1898) and Haemonchus bispinosus (Molin, 1860) Railliet and Henry (1909), with a note on the synonymy of Haemonchus placei (Place, 1893) Ransom (1911) with H. bispinosus. *Indian Journal of Helminthology*, *16*(1), 5-11.

Sangma, A., Begum, N., Roy, B. C., & Gani, M. O. (2012). Prevalence of helminth parasites in sheep (Ovis aries) in Tangail district, Bangladesh. Journal of the Bangladesh Agricultural University, 10(452-2016-35654), 235-244.

Santos, M. C. D., Amarante, M. R. V., Silva, M. R. L. D., & Amarante, A. F. T. D. (2014). Differentiation of Haemonchus placei from Haemonchus contortus by PCR and by morphometrics of adult parasites and third stage larvae. *Revista Brasileira de Parasitologia Veterinária*, *23*(4), 495-500.

Sato, M. O., Sato, M., Chaisiri, K., Maipanich, W., Yoonuan, T., Sanguankiat, S., ... & Waikagul, J. (2014). Nematode infection among ruminants in monsoon climate (Ban-Lahanam, Lao PDR) and its role as food-borne zoonosis. *Revista Brasileira de Parasitologia Veterinária*, *23*(1), 80-84.

Shahnawaz, M., Shahardar, R. A., & Wani, Z. A. (2011). Seasonal prevalence of platyhelminthosis of sheep in Ganderbal area of Kashmir valley. *Journal of Veterinary Parasitology*, *25*(1), 59-62.

Sharkhuu, T. (2001). Helminths of goats in Mongolia. *Veterinary Parasitology*, *101*(2), 161-169.

Sharma D. K., Agrawal N., Mandal A., Nigam P. and Bhushan S., (2009). Coccidia and gastrointestinal nematode infections in semi-intensively managed Jakhrana goats of semi-arid region of India, Tropical and Subtropical Agroecosystems, 11, 135- 139.

Shirale, S.Y. and Made, D.K. (2007) Bionomics of helminth parasites in goats, Indian Vet. J., 84, 1237-1239.

Singh, A. K., Das, G., Roy, B., Kumar, S., Naresh, R., & Nath, S. (2015). Prevalence of gastrointestinal nematodes of goat on communal pasture in Madhya Pradesh. *Indian Vet. J*, *92*(3), 67-68.

Singh, A. K., Das, G., Roy, B., Nath, S., Naresh, R., & Kumar, S. (2015). Prevalence of gastro-intestinal parasitic infections in goat of Madhya Pradesh, India. *Journal of Parasitic Diseases*, *39*(4), 716-719.

Singh, K. S. (2003). Veterinary helminthological. New Delhi: ICAR. pp., 354–366.

Sissay M. M., Uggla A. & Waller P. J. (2007). Prevalence and seasonal incidence of nematode parasites andfluke infections of sheep and goats in easternEthiopia. Tropical Animal Health and Production 39, 521-531.

Skerman, K.D. & Hillard, J.J.(1966). A hand book for studies of helminth parasites of ruminants (N.E.A.H.I. hand book No.2). Food and Agricultural Organization of the united Nation.

Smith, G., & Grenfell, B. T. (1994). Modelling of parasite populations: gastrointestinal nematode models. *Veterinary Parasitology*, *54*(1-3), 127-143.

Sood M L (1981) Haemonchus in India. Parasitol. 83:639-650

Soulsby, E. J. L. (1982). Helminths. *Arthropods and Protozoa of domesticated animals*, *291*.

Soulsby, E. J. L., (1986). Helminth, Arthropods and Protozoa of domesticated animals. pp 212-242.

Soulsby, E.J.L (1965). Textbook ofVeterinary Clinical ParasitologyVolume 1. Helminths. BlackwellScientific Publications, Oxford. 1120pp.

Stevenson, L. A., Chilton, N. B., & Gasser, R. B. (1995). Differentiation of Haemonchus placei from H. contortus (Nematoda: Trichostrongylidae) by the

ribosomal DNA second internal transcribed spacer. *International journal for parasitology*, *25*(4), 483-488.

Sultan, K., Elmonir, W., & Hegazy, Y. (2016). Gastrointestinal parasites of sheep in Kafrelsheikh governorate, Egypt: Prevalence, control and public health implications. *Beni-Suef University Journal of Basic and Applied Sciences*, *5*(1), 79-84.

Susan, G. W. and Barbara, J. F. (2009). *Veterinary Herbal Medicine* (3): 714.

Sutar, A. U., Kengar, S. B., Patil, S. S. & Khan, M. R. (2010). Prevalence of Gastrointestinal Parasites in Goats of Ahmednagar district of Maharashtra. *Vet. World3* (10):456-457.

Sweeny, J. P. A., Robertson, I. D., Ryan, U. M., Jacobson, C. & Woodgate, R. G. (2011). Comparison of molecular and McMaster techniques to confirm the presence of naturally acquired strongylid nemato de infections in sheep. *Molecular and Biochemical Parasitology*, 180, 62-67.

Sykes, A. R. (1994). Parasitism and production in farm animals. *Animal Science*, *59*(2), 155-172.

Talukdar, S. K., (1996). Prevalence of Helminthic infection in goats in Assam. *Journal of Veterinary Parasitology.*, 10: 83–86.

Tamura K, Dudley J, Nei M and Kumar S (2007). Molecular evolutionary genetics analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.*, 24, 1596-1599.

Tan, T. K., Panchadcharam, C., Low, V. L., Lee, S. C., Ngui, R., Sharma, R. S., & Lim, Y. A. (2014). Co-infection of Haemonchus contortus and Trichostrongylusspp. among livestock in Malaysia as revealed by amplification and sequencing of the internal transcribed spacer II DNA region. *BMC veterinary research*, *10*(1), 38.

Tariq, K. A., Chishti, M .Z. & Ahmad, F. (2010) Gastro- intestinal nematode infections in goats relative to season, host, sex and age from the Kashmir valley, *India. J. Helminthol.* 84, 93–97.

Tavares, R. G., Staggemeier, R., Borges, A. L. P., Rodrigues, M. T., Castelan, L. A., Vasconcelos, J., ... & Spalding, S. M. (2011). Molecular techniques for the study and diagnosis of parasite infection. *Journal of Venomous Animals and Toxins including Tropical Diseases*, *17*(3), 239-248.

Taylor, M. A., Coop, R. L., & Wall, R. L. (2007). Parasites of dogs and cats. *Veterinary parasitology*, *3*, 429-430.

Taylor, M. A., Coop, R. L., & Wall, R. L. (2007). Text book of veterinary parasitology 3rd edition Blackwell publishing Ltd.

Teklemariam, T., Fantahun, T., Alemayehu, B., & Beyene, A. (2016). Efficacy of selected anthelmintics against gastrointestinal nematodes on naturally infected small ruminants in Gimbo district, Kaffa Zone, South West Ethiopia. *Livestock Research for Rural Development*, *28*, 12.

Temesgen, T. (2008). Study on prevalence of ovine gastrointestinal parasite in and around Bedelle. *DVM thesis, Haramaya University, Faculty of Veterinary Medicine, Haramaya, Ethiopia*.

Tigist, K., Jemere, B. & Desie, Sh. (2015). Gastrointestinal nematodes of sheep and goats in three districts of Kaffaand Bench Maji Zone, South West, Ethiopia. Vet. J19 (2): 67-76.

Tod, E. (1965). On the morphology of Haemonchus contortus (Rudolphi) Cobb (Nematoda: Trichostrongylidae) in sheep and cattle. *Australian Journal of Zoology*, *13*(5), 773-782.

Uddin, M. Z., Farjana, T., Begum, N., & Mondal, M. M. H. (2006). Prevalence of amphistomes in Black Bengal goats in Mymensingh district. *Bangladesh Journal of Veterinary Medicine*, *4*(2), 103-106.

Umur, Ş., & Yukari, B. A. (2005). Seasonal activity of gastro-intestinal nematodes in goats in Burdur region, Turkey. *Turkish Journal of Veterinary and Animal Sciences*, *29*(2), 441-448.

Uriarte, J., Llorente, M. M., & Valderrábano, J. (2003). Seasonal changes of gastrointestinal nematode burden in sheep under an intensive grazing system. *Veterinary Parasitology*, *118*(1-2), 79-92.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M. & Jennings, F.W. (1996).Veterinary Parasitology. Second Edition, Blackwell Science Ltd, Oxford,U.K. Blackwell Publishing Company.

Vadlejch, J., Lukešová, D., Vašek, J., Vejl, P., Sedlák, P., Čadková, Z., & Salaba, O. (2014). Comparative morphological and molecular identification of Haemonchus species in sheep. *Helminthologia*, *51*(2), 130-140.

Vadlejch, J., Lukešová, D., Vašek, J., Vejl, P., Sedlák, P., Čadková, Z., ... & Salaba, O. (2014). Comparative morphological and molecular identification of Haemonchus species in sheep. *Helminthologia*, *51*(2), 130-140.

Valcarcel, F. & Garcia Romero, C. (1999).Prevalence and seasonal pattern ofcaprine trichostrongyles in a dry area ofcentral Spain. Journal of VeterinaryMedicine Series B 46(10): 673–681.

Van Dijk, J., David, G. P., Baird, G., & Morgan, E. R. (2008). Back to the future: developing hypotheses on the effects of climate change on ovine parasitic gastroenteritis from historical data. *Veterinary parasitology*, *158*(1-2), 73-84.

Van Wyk, J. A., & Mayhew, E. (2013). Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. *Onderstepoort Journal of Veterinary Research*, *80*(1), 00-00.

Van Wyk, J. A., Stenson, M. O., Van der Merwe, J. S., Vorster, R. J. and Viljoen, P. G., (1999) Anthelmintic resistance in South Africa: Surveys indicates an extremely serious situation in sheep and goat farming. *Onderstepoort Journal of Veterinary Research*, 66, 273-284.

Vlassoff, A., Leathwick, D. M., & Heath, A. C. G. (2001). The epidemiology of nematode infections of sheep. *New Zealand Veterinary Journal*, *49*(6), 213-221.

Waller, P. J., 1997. Anthelmintic resistance. Veterinary Parasitology, 72, 391-412.

Waruiru, R.M., Otieno. R.O. and Mutune, M.N. (2005). Gastro-intestinal parasitic infections of sheep and goats in semi-arid areas of Machakos district, Kenya. Bulletin of animal health and production in Africa 53: 25-34.

Wilson, A., Mellor, P., Bluetongue (2007). Vectors, epidemiology and climate change. Parasitol. Res., 103, 69-77.

Xiang, Z., Chen, X., Yang, L., He, Y., Jiang, R., Rosenthal, B. M., ... & Yang, Z. (2009). Non-invasive methods for identifying oocysts of Sarcocystis spp. from definitive hosts. *Parasitology international*, *58*(3), 293-296.

Yadav, A., Khajuria, J. K., & Raina, A. K. (2006). Seasonal prevalence of gastrointestinal parasites in sheep and goats of Jammu. *Journal of Veterinary Parasitology*, *20*(1), 65-68.

Yamaguti, S. (1961). Systema helminthum. Volume III. The nematodes of vertebrates. *Systema helminthum. Volume III. The nematodes of vertebrates.*

Yasin, U., Wodajnew, B. & Tsehaine, D. (2017). Study on the Prevalence of GIT Nematode Infection of Small Ruminants in Kurmuk Woreda, Assosa Zone of Benishangul Gumuz Region, Western Ethiopia. *Report and Opinion* ;9(10). *76*(5): 435-437.

Yeasmin, T., Khanum, H., & Zaman, R. F. (2014). Seasonal prevalence of arthropoda and helminth parasites in sheep (Ovis aries. *Bangladesh Journal of Zoology*, *42*(1), 45-55.

Yevstafieva, V. A., Kravchenko, S. O., Gutyj, B. V., Melnychuk, V. V., Kovalenko, P. N., & Volovyk, L. B. (2019). Morphobiological analysis of Trichuris vulpis (Nematoda, Trichuridae), obtained from domestic dogs. *Regulatory Mechanisms in Biosystems*, *10*(2), 165-171.

Zahida, M. (1992). Helminth parasites of vertebrates of Leh(Ladakh). M.Phil. dissertation, Department of Zoology, University.

Zainab, T., & Khan, W. (2016). Morphological and histopathological studies of Trichuris ovis in goat intestine. *J. Parasit. Dis. Diag. Ther*, *1*, 122-129.

Zaman, M. A., Iqbal, Z., Khan, M. N., & Muhammad, G. (2012). Anthelmintic activity of a herbal formulation against gastrointestinal nematodes of sheep. *Pak. Vet. J*, *32*(1), 117-121.

Zeryehun, T., (2012). Helminthosis of sheep and goats in and around Haramaya, Eastern, Ethiopia. *J. Vet. Med. and Anim. Health 4*(3): 48-55.

Zvinorova, P. I., Halimani, T. E., Muchadeyi, F. C., Matika, O., Riggio, V., & Dzama, K. (2016). Prevalence and risk factors of gastrointestinal parasitic infections.

# Appendix

*Appendix -1*

PAPERS PUBLISHED IN JOURNALS

| S.NO | TITLE OF THE PAPER | AUTHORS | JOURNAL NAME/VOLUME (ISSUE)/PAGE NUMBER | YEAR |
|---|---|---|---|---|
| 1. | STUDIES ON PREVALENCE OF GASTROINTESTINAL PARASITIC INFECTION IN GOAT (*CAPRA HIRCUS*) IN LUCKNOW (UTTAR PRADESH) | KAMAL JAISWAL, SUMAN MISHRA AND SAVITA | JOURNAL OF EXPERIMENTAL ZOOLOLY INDIA | 2020 |
| 2. | MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *OESPHAGOSTOMUM COLUMBIANUM* IN GOAT AT LUCKNOW DISTRICT, UTTAR PRADESH | KAMAL JAISWAL, SUMAN MISHRA AND SAVITA | BIOCHEMICAL AND CELLULAR ARCHICHES | 2020 |

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# STUDIES ON PREVALENCE OF GASTROINTESTINAL PARASITIC INFECTION IN GOAT (*CAPRA HIRCUS*) IN LUCKNOW (UTTAR PRADESH)

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**ABSTRACT : Goat rearing is an important part of Livestock in India, which is economically important through milk and meat products to the farmers and also contributes for the national and international economy. Parasitism is among the major problems faced by farmers. Nematodes (Helminth) are most prevalent parasites following the cestode and trematode, instudy area. The study was carried out from May 2016 to April 2017 in Lucknowto estimate the prevalence in accordance to the month, age and gender-wise. During the study period, 180 gastrointestinal tracts, collected from randomly dissected and presence of parasitic infection was confirmed. The study revealed that nematodes were identified in goats with overall prevalence of 63.33% (n = 180) across various age groups (36.84%, 27.19%, 35.08%, in > 6 month, between 6 -12 month and < 12 month), respectively. The prevalence was found to be among male and female respectively 46.49% and 53.50%.**

***Key words :*** Goat, gastrointestinal tract, helminth parasites, prevalence.

## INTRODUCTION

Small ruminants are important source of employment for rural communities around the world as well as being socially (Kelly *et al*, 2012) and economically highly significant at national and international levels, as with all livestock species (Muluneh *et al*, 2017). In India, 70% rural households depend on animal protein and primarily raised for milk, meat, hair and leather (Kaur *et al*, 2015; Nakanishi *et al*, 2011; Tariq *et al*, 2010*,* and Nwosu CO *et al*, 2007), goat havethe most important role amongst the small ruminant (Mir *et al*, 2013) where India has second largest goat population (117 million) and goat meat production (125.7 million) in the world (FAO, 2008). The effort of these to gross national product is approximately 47300 million per year (Honmade, 1998). The amount of meat production on an average is 0.5% in India 15% in Uttar Pradesh (Singh *et al*, 2015). The small ruminants have benefited little from vast resource owning to anassembly of difficulties, diseases are most dangers for the health of goats (Demelash *et al*, 2006). The major constraint, which leads to large economic losses attributable to reduced weight gain, retarded growth and decreasedp roduction of goat farming (Jas and Ghosh, 2009).

Helminth infections are one of the biggest problems among the small ruminants and health problems, which has a major effect on the animal's performance and cause great economic losses to the producer (Biu *et al*, 2006 and FAO, 2002). Although, parasites isserious problem in the developing world, particularly where nutrition and sanitation are poor (Palanivel *el al*, 2012).

World-wide, more money is spent on prevention of diseases from internal parasites among small ruminants (Bath *et al*, 2000).

Nematodes are most prevalent than the cestode and tremotode parasites (Badran *et al*, 2012). Prevalence of gastrointestinal nematode parasites has been reported ranging from 72.0 to 84.1% in domestic animals from various parts of the World (Bundy *et al*, 1983). Short-term patterns in parasite infection and comprehensive knowledge in order to devise an appropriate method in small ruminants due to the associated morbidity, mortality and cost-effective strategy to control this parasite (O'Connor *et al*, 2007; Sutar *et al*, 2010; Rahman *et al*, 1975; Morgan *et al*, 2005; Bukhari *et al*, 2011; Singh *et al*, 2013). The losses due to diseases in goats scaled at national level were estimated at Rs. 11,720 million per annum (FAO, 2008). They cause retarded growth, lowered productivity, mortality (Soulsby, 1982) and high economic losses (Iqbal *et al*, 2007), thus affecting the income of management and small holders of dairy farming communities (Khan *et al*, 2010). In India, the population

of goat was 27% respectively of the total livestock population (Anonymous, 2012). The agro-ecological and geo-climatic conditionsalso contribute to the growth and multiplication of parasitic diseases in goats (Haq *et al*, 1968).

## MATERIALS AND METHODS

The study was conducted from May 2016 to April 2017 around in Lucknow district. The climatic condition of this study area is mainly depend on temperature and humidity. (June and July are hottest months) (av. max. and mini. temp. of 40°C and 12 °C temp., respectively) whereas December and January are the coldest month (av. max. and mini. temp. of 12°C and 4°C, respectively).

The study was carried out on a total number of 180 goats, which are in local breed, conventionally, host were demarcated as kid, young and adult. The gastrointestinal tracts of host from different regions of Lucknow were collected and brought to the Zoology lab of Babasaheb Bhimrao Ambedkar University, Lucknow for detection of parasites. Collected parasites were preserved in glycerol-alcohol (3:1), mounted in glycerol and images were taken using by compound microscope. An identification technique was done by Soulsby key (1982).

Statistical analysis of Datawas done by $\chi^2$ – test with helpof software SPSS version -20. Explanatory statistics was used to determine, association between categorical factors such as month, age and gender and relative prevalence was calculated using the formula as specified by Margolis *et al* (1982).

$$\text{Prevalence}\ (\%) = \frac{\text{Total number of individuals infected with a particular parasite species}}{\text{Total number of hosts examined}} \times 100$$

## RESULTS

Total 180 gastrointestinal tracts were examined during the study period (2016 May to 2017 April) in which 140 (63.33%) were found to be observed infected. The highest rate of infestation was found during August (100%) followed by September (93.33%) and lowest parasitic infection was found in January (13.33%) (Table 1). $\chi^2$ – test revealed significant difference between prevalence during all months (≤ 0.05 significant).

The age wise study of the goat was an important aspect which influences the prevalence of gastrointestinal parasites. The host'sage were categorized in to three groups based on their age as kid (group I), young (group II) and adult (group III). Highest prevalence was recorded in the age group I(> 6 month) in which 43 out of 114 tracts were observed infested with parasites (36.84%). The second prevalence was found inage group II (6-12 month), where 31 tracts out of 114 were found to be infected (27.19%). The infestation was observed in age group III (<12 month) in which 40 tracts out of 114 were found infested with parasitic infection (33.09%) (Table 2). Non-significant differences in infected intestine on basis of age group (≥.05 non-significant) difference among (Table 2).

The gender-wise experienced non-significant (≥.05) differences infection in many goat was found and represented in Table 3. The result showed that females (53.50%) were more infected as compared to male goats (46.49%). It may be due to the stall feeding, hormonal differences and stress during pregnancy in females.

**Table 1 :** Month-wise prevalence of nematode parasites.

| S.no. | Months (2016-2017) | No. of hosts examined (n=180) | | Prevalence |
|---|---|---|---|---|
| | | Total no. of intestine | Infected intestine (114) | |
| 1. | May (2016) | 15 | 13 | 86.66% |
| 2. | June (2016) | 15 | 13 | 86.66% |
| 3. | July (2016) | 15 | 14 | 93.33 % |
| 4. | August (2016) | 15 | 15 | 100.00 % |
| 5. | September(2016) | 15 | 14 | 93.33 % |
| 6. | October(2016) | 15 | 13 | 86.66% |
| 7. | November(2016) | 15 | 09 | 60.00% |
| 8. | December(2016) | 15 | 04 | 26.66% |
| 9. | January(2017) | 15 | 02 | 13.33% |
| 10. | February(2017) | 15 | 03 | 20.00% |
| 11. | March(2017) | 15 | 05 | 33.33% |
| 12. | April(2017) | 15 | 09 | 60.00% |

($\chi^2$ = 73.78 and p value = 2.32E-11 (≤ 0.05 significant).

**Table 2 :** Season -wise prevalence of nematode parasites of goat.

| S. no. | Age group | Total no. of examined intestine/infected intestine | Prevalence |
|---|---|---|---|
| 1 | Kid (> 6 month) | 43/114 | 36.84% |
| 2 | Young (Between 6 monthsto 12 month) | 31/114 | 27.19% |
| 3 | Adult (<12 month) | 40/114 | 35.08% |

$\chi^2$ = 3.08 and p value = 0.214 (≤ .05 non-significant).

**Table 3 :** Gender-wise prevalence of nematode of goat.

| S. no. | Gender | Total no. of examined intestine/infected intestine | Prevalence |
|---|---|---|---|
| 1. | Male | 53/114 | 46.49% |
| 2. | Female | 61/114 | 53.50% |

$\chi^2$ = 1.12 and p value = 0.289 (≥ .05 non-significant).

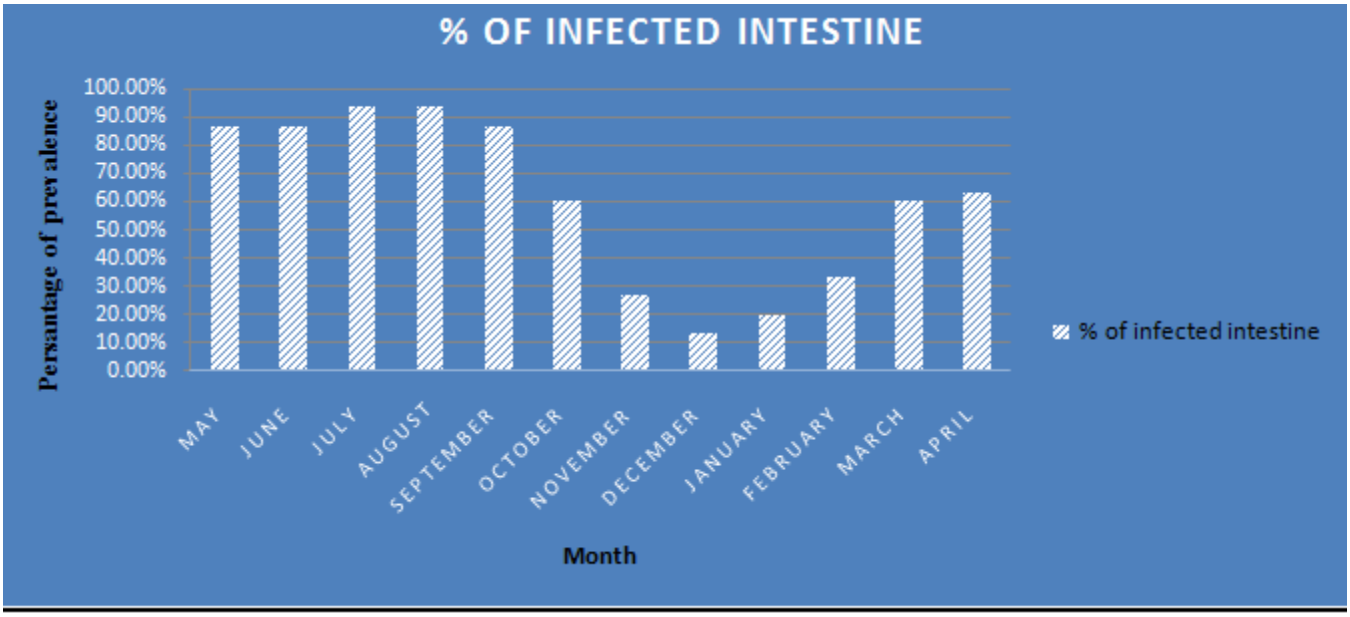


**Fig. 1 :** Month-wise prevalence of nematodes parasites of goat.

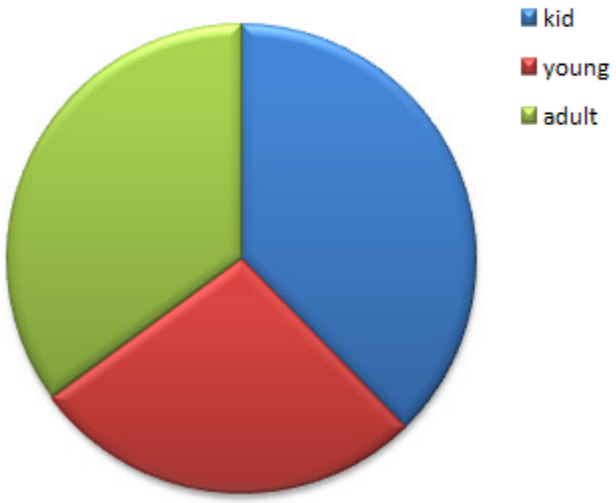


**Fig. 2 :** Age wise prevalence of nematodes parasites.

## DISCUSSION

The overall prevalence of gastrointestinal parasites of goats werefound to be 63.33%, during the 12 months (May, 2016 to April, 2017), collected from the gastrointestinal tracts of goat. The hot and humid climate of study area favours the development of gastrointestinal parasite on pasture (Enyenihi *et al*, 1975 and Hawlader *et al*, 2002) and also depends on the rainfall of the particular area (Raza *et al*, 2007). August and September have higher parasitic infection compared to December to February, similar findings were reported by many researcher (Nakanishi *et al*, 2011; Shirale *et al*, 2007; O'Connor *et al*, 2007 and Skykes, 1994).

Age-wise prevalence revealed that the overall gastrointestinal parasitic infection was slightly higher in kid (36.84%) than in young goats (27.19%) and adults

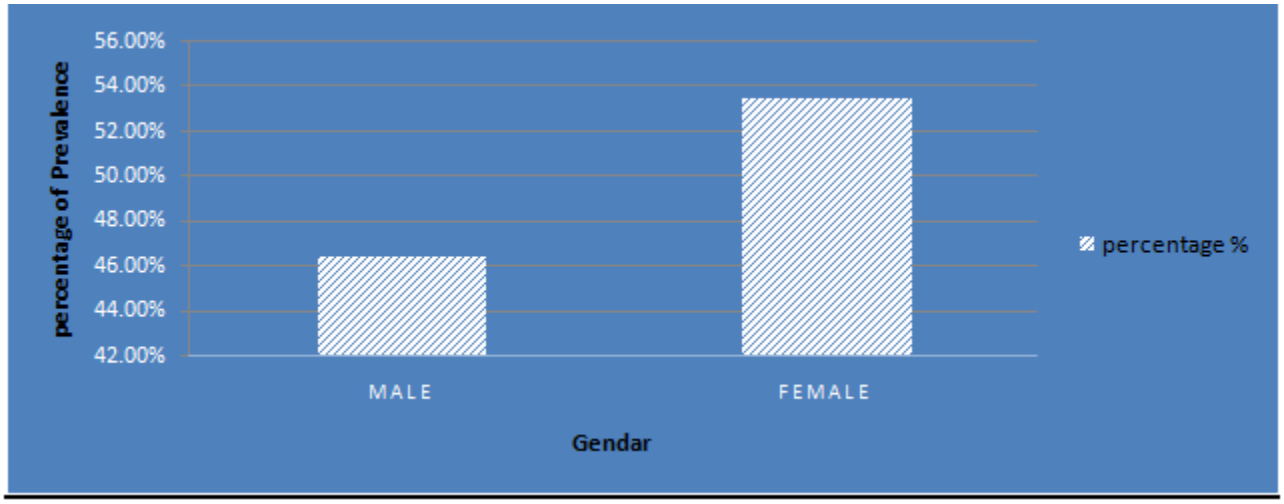


**Fig. 3 :** Gender wise prevalence of nematodes parasites.

(35.08%) as in reported by Shah *et al* (2015) and Das *et al* (2015). However, much difference prevalence of nematode parasites in kids and adults was not found and the finding are at with the report of Singh *et al* (2015). The parasiticwas found to be infestation higher in kid and adult than the young and this may be due to the reason that the kid and adult goat have acquired immunity to parasites through frequent challenges and expel the ingested parasites before they establish infections (Shah-Fischer *et al*, 1989 and Dun, 1978). Sharma *et al* (2009) and Emiru *et al* (2013), also reported that the kids are more susceptible as compared to adults and worm burdens of worm decrease with increasing age due to immunological maturity acquired after repeated exposure and almost similarity finding were reported by many researchers (Sissay *et al*, 2006 and Laksmi *et al*, 2001).

Prevalence of infection was found to be highly in female goats than the male. The presence of sex difference in infection is also conflicted with other reports of Ghanem *et al* (2009) and Hassan *et al* (2013). Higher prevalence of gastrointestinal parasites in females may be, due to the stall feeding of female animals during pregnancy, which reduces exposure to pasture contamination and normally females are assumed to be more heavily infested due to hormonal differences and stress during pregnancy (Muluneh *et al*, 2014; Dagnachew, 2011 and Ayaz *et al*, 2013).

## CONCLUSION

Helminthiasis parasitic infection is one of the major constraints in goat industry revealed contributing to maximum in economic lossesin Lucknow (Uttar Pradesh). The present study conducted that various Helminth parasites were found in different breed of goats, which is the most prevalent disease. Some control measures for gastrointestinal parasites can be undertaken to reduce the intensity of the infection. In this regard, it is suggested that practice of separate grazing of an animal with low stocking rate and proper deforming at regular intervals may be adopted. New technology should be adopted for the diagnosis of parasites.

## REFERENCES

Anonymous (2012) Department of animal husbandry, dairying and fisheries. Ministry of Agriculture, Government of India. pp.1-180.

Ayaz M M, Raza M A, Murtaza S and Akhtar S (2013) Epidemiological survey of helminths of goats in southern Punjab, Pakistan. *Trop. Biomed*. **30**, 62-71.

Badran I R, Abuamsha R, Aref W, Alqisi and Alumor J (2012) Prevalence and Diversity of gastrointestinal parasites in small ruminants under two different rearing systems in Jenin district of Palestine. Department of Animal Resources, National Centre for Agricultural research, Ministry of Agriculture, Palestine. *An-Najah University J. Res. - Natural Sci.* **26**, 1-18.

Bath G F and Wet J D (2000) *Sheep and goat diseases*. Tafelburg Publishers Ltd., Cape Town, South Africa.

Biu A A and Eteng F O (2006) Some parasites causing diarrhea amongst kid goats in Maiduguri, Nigeria. In: *Proceedings of the 6th Annual Conference of Animal Science Association of Nigeria*. September 17th -19th, 2001 University of Maiduguri Convocation Square, 232-233.

Bukhari S and Sanyal P K (2011) Epidemiological Intelligence for GrazingManagement in Strategic Control of Parasitic Gastroenteritis in Small Ruminants in India. *Vet. World* **42**, 92-96.

Bundy D A P, Arambulo P V and Grey C L (1983) Fascioliasis in Jamaica: epidemiologic and economic aspects of a snail-borne parasitic zoonosis. *Bull. Pan. Am. Hlth. Org*. **17**, 243–258.

Chattopadhyay A K and Bandyopadhyay S (2013) Seasonal variations of EPG Levels in gastro-intestinal parasitic infection in a Southeast Asian controlled locale: a statistical analysis. SpringerPlus **2**, 205.DOI: https://doi.org/10.1186/2193-1801-2-205

Dagnachew S, Amamute A and Temegen W (2011) Epidemiology of gastrointestinal helminthiasis of small ruminants in selected sites of North Gondar zone, Northwest Ethiopia. *Ethiopian Vet. J.* **15**, 57-68.

Demelash B, Yilma J and Hassen C (2006) Ovine helminthosis is major health constraints to productivity of sheep in Ethiopia, Faculty of Veterinary Medicine, Awassa University, Awassa, Ethiopia. pp. 65-90.

Enyenihi U K, Okon E D and Fabiyi J P (1975) Tapworm infection of small ruminrnt in Nigeria. *Bull. Hlth. Prod. (Africa)* **23**(3), 289-295.

Emiru B, Amede Y, Tigre W, Feyera T and Deressa B (2013) Epidemiology of gastrointestinal parasites of small ruminants in Gechi District, Southwest Ethiopia. *Adv. Biol. Res.* **7**, 169-174.

FAO (2008) Distribution and impact of helminth diseases of livestock in developing countries. FAO Animal Production and Health Paper. Vol. **96**. FAO, Rome, 221.

Food and Agriculture Organization (FAO) (2002) Biological control of nematode parasites of small ruminants in Asia. *FAO Animal Prod. Health Paper* **20**, 1-100.

Food and Agriculture Organization (FAO) (2010) Breeding strategies for sustainable management of animal genetic resources. Animal Production and Health Guidelines, No.3. Food and Agriculture Organization of the United Nations, Rome, Italy.

Ghanem Y M, Naser M H, Abdelkader A H and Heybe A (2009) An Epidemio coprological study of protozoan and nematode parasites of ruminants in tropical semi-arid district of Somalilan D (North of Somalia). *Veterinary Medical Journal*. 3rd Science Congress, pp. 768-787.

Haq S and Shaikh H (1968) A surveys of helminth parasiting the gastro-intestinal tracts of goats and sheep in East Pakistan. *East Pakistan J. Vet. Sci.* **2**, 54.

Hassan D I, Mbap S T and Naibi S A (2013) Prevalence of Worm Infection in Yankasa sheep and West African dwarf goats in Lafia Town and Environs, Nigeria. *IOSR J. Agricult. Vet. Sci.* **4**(4), 84-90

Hawlader M M R, Mahbub-E-Elahi A T M, Habib S and Bhuyan M

J U (2002) Gastro-intestinal nematode infestations in the black Bengal goats of Sirajgong district of Bangladesh. *J. Biol. Sci*. **2**, 556-557.

Honmade (1998) Factor like breeding, feeding and management and disease control affect the meat potential.

Iqbal Z, Jabbar A and Yaseen M (2007) Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. *J. Helminthol.* **81**, 323–328.

Jas R and Ghosh J D (2009)Economic impact of gastrointestinal nematodosis in sheep: enhanced meat production by anthelmintic treatment. *Indian J. Animal Sci.***79**(8), 3-5.

Kaur D, Jaiswal K and Mishra S (2015) Studies on prevalence of Ixodid ticks infesting cattle and their control by plant extract. *IOSR J.Pharm. Biol. Sci.* **10** (6), Ver. III, 1-11.

Kelly G A, Walkden-Brown S W and Kahn L P (2012) No loss of production due to larval challenge in sheep given continuous anthelmintic treatment via a controlled release capsule. *Vet. Parasitol*. **183**, 274–283.

Khan M N, Sajid M S, Khan M K, Iqbal Z and Hussain A (2010) Gastrointestinal helminthiasis: prevalence and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan. *Parasitol. Res*. **107**, 787–794.

Laksmi H D, Jagannath M S and D'Souza P E (2001) Gastrointestinal parasitic infections in sheep atdifferent forms of Karnataka. *J. Vet. Parasitol.* **15**, 133-135.

Maqsood M, Iqbal Z and Chaudhry A H (1996) Prevalence and intensity of haemonchosis with reference to breed, sex and age of sheep and goats. *Pakistan Vet. J*. **16**(1), 41–43.

Mir M R, Chishti M Z, Dar S A and Rashid M (2013) Bionomics of Helminth Parasites in Goats of Subtropical Jammu Area of J & K, India. *Agric. Biol. J. N. Am*. **4**(3), 316-319.

Morgan E R, Cavill L, Curry G E, Wood R M and Mitchell (2005) Effects of aggregation and sample size on composite faecal egg counts in sheep. *Vet. Parasitol.* **131**, 79-87.

Muluneh J, Bogale B and Chanie M (2017) Major Gastrointestinal Nematodes of Small Ruminants in Dembia District, Northwest Ethiopia. *Europ. J. Appl. Sci.* **6**, 30-36.

Nabi H, Saeed K, Shah S R, Rashid M I, Akbar H and Shehzad W (2014) Epidemiological study of gastrointestinal nematodes of goats in district Swat, Khyber Pakhtunkhwa, Pakistan. *Sci. Int*. **26**(1), 283–286.

Nakanishi Y, Takayama K and Yasuda N (2011) Effects of Japanese Bead-tree (*Melia azedarach* var. *subtripinnata*) on Gastrointestinal Parasites in Goats. *JARQ* **45**(1), 117- 121.

Nwosu C O, Madu P P and Richards W S (2007) Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi- arid zone of north-eastern Nigeria. *Vet. Parasitol*. **144**(1-2), 118-124.

O'Connor L J, Kahn L P and Walkden-Brown S W (2007) Moisture requirements for the free-living. **142**(1-2), 1-15.

Odoi A, Gathuma J M, Gachuiri C K and Omore A (2000) Risk factors of gastrointestinal nematode parasite infections in small ruminants kept in smallholder mixed farms in kenya. *BMC Vet. Res.* **3**, 1746-6148.

Palanivel K M, Rishikesavan R, Sureshkumar K and Gopi H (2012) Epidemiology of parasitic infection in Madras red sheep in Tamil Nadu. *Int. J. Food Agric. Vet. Sci*. **2**(3), 140-145.

Rahman M H, Soliman K N and Shaikh H (1975) Proceedings of Pakistan Scientific Conference, University of Peahwar. **17**.

Raza M A, Iqbal Z, Jabbar A B and Yaseen M (2007) Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. *J. Helminthol*. **81**, 323-328.

Regassa F, Teshale S, Reta D and Yosef K (2007) Epidemiology of gastrointestinal parasites of ruminants in Western Oromia, Ethiopia. *Int. J. Appl. Res. Vet. Med.* **4**, 51-57.

Rossanigo C E and Grunder L (1995) Moisture and temperature requirements in feces for the development of free-living stages of gastrointestinal nematodes of sheep and cattle and deer. *J. Helminthol.* **67**, 357-362.

Sharma D K, Agrawal N, Mandal A, Nigam P and Bhushan S (2009) Coccidia and gastrointestinal nematode infections in semi-intensively managed Jakhrana goats of semi-arid region of India. *Tropical and Subtropical Agroecosystems* **11**, 135- 139.

Sissay M M, Uggla A and Waller P J (2007) Epidemiology and seasonal dynamics of gastro-intestinal infections of sheep in a semi-arid region of eastern Ethiopia. *Vet. Parasitol*. **143**, 311-321.

Singh V, Dash S K and Lal H P (2013) Prevalence of gastrointestinal parasites in sheep and goats in and around Mathura, India. *Vet. World* **6**(5), 260-262.

Singh A K, Das G, Roy B, Nath S, Naresh R and Kumar S (2015) Prevalence of gastro-intestinal parasitic infections in goat of Madhya Pradesh, India. *J. Parasit. Dis.* **39**, 716-719.

Skykes A R (1994) Parasitism and production in farm animals. *Animal Parasitology* **59**, 155-172.

Soulsby E J L (1982) Helminths, Arthropods and Protozoa of Domesticated Animals.London: Bailliere Tindal, 809 pp. + illustations.

Sutar A U, Kengar S B, Patil S S and Khan M R (2010) Prevalence of Gastrointestinal Parasites in Goats of Ahmed Nagar district of Maharashtra. *Vet. World* **3**(10), 456-457.

Tariq K A, Chishti M Z and Ahmad F (2010) Gastro- intestinal nematode infections in goats relative to season, host, sex and age from the Kashmir valley, India. *J. Helminthol.* **84**, 93–97.

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# MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *OESPHAGOSTOMUM COLUMBIANUM* IN GOAT AT LUCKNOW DISTRICT, UTTAR PRADESH

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**ABSTRACT : *Oesphagostomum* species (nodular worm) is a most infectious parasite in tropical and sub-tropical area in the world. It is found in the large intestine of goat. The study was done with the help of morphological and molecular characterization of *Oesphagostomum spp.* Adult nematodes parasites were collected from gastrointestinal nematode parasites of goat freshly collected from the different slaughter houses of the Lucknow, Uttar Pradesh. Samples were morphologically identified by following standard key however, for molecular study, *Oesphagostomum* species were isolated and genetically identified by second internal transcribed spacers of ribosomal (ITS2) region. The amplified product was observed size of band (117bp). Phylogeny analysis was observed 98% similarity by UPGMA method compared with the sequences of accession number.**

***Key words :*** *Oesphagostomum columbianum*, morphology, PCR, phylogeny.

## INTRODUCTION

*Oesophagostomum*, or nodule worm is a nematode parasite belonging to the family Chabertiidae (Anderson, 2000). Infection with these helminthes can cause nodule in the walls of the small and large intestines (Baron and Wakelin, 1996). They are responsible for losses in growth and productivities in the goat industries. Various optical conditions are responsible for the parasitic infection for the development, survival and spreading of the parasites (Odoi *et al*, 2007 and Rahman, 1994). Some major causing clinical signs are anorexia, bloody diarrhea (Wilmsen *et al*, 2014) weight loss, emaciation, hypo proteinaemia, poor body condition, malnutrition and death in the case of heavy parasitic burden (Holmes, 1985; Hale, 2006 and Taylor *et al*, 2007). This parasitic are play a major role in loss of production and reduce income of farmer in the world (Hoste *et al*, 1995). Therefore, effective prevention and control of gastrointestinal parasitic diseases are important for the host.

The identification of this parasite has been based on morphological and molecular characterization method. Morphological identification of parasites has been described by light-microscopy, based on total body length, spicules, Corona radiata external and internal composed of a buccal leaves (Goodey, 1924; Chitwood, 1931; Soulsby, 1965; Skryabin *et al*, 1992; Blot-kamp *et al*, 1993 and Hartwich, 1994).

Molecular characterization is used as a diagnostics tools since 1990s for the identification, quantification, discrimination of the parasitic diseases. By the diagnosis methods, we can easily detect the parasitic infection (Ahmad M Singh, 2011). This technique is a accurate, alternative diagnostic approaches and given data about genetic variation (genome- species specific) by PCR, DNA sequencing and bioinformatics (Gasser *et al*, 1993; Karp *et al*, 1996; Gasser, 2006; Punja *et al*, 2008 and Tan *et al*, 2014). The ITS2 (second transcribed spacer 2) sequences can be used as a tool for species differentiation in gastrointestinal nematodes (Stevenson *et al*, 1995).

Sequences of the ITS2 (Internal Transcribed Spacer) of rDNA have been widely used as genetic markers for the differentiation of many nematodes and identification (Gasser and Newton, 2000 and Lin *et al*, 2012). This marker has been generally used to explain the classification and molecular relationships of parasites (Dorris *et al*, 2002; Marigo *et al*, 2011; Anderson *et al*, 2012). This study also conclude the homology of *Oesophagostomum* species was described by other accession no. of parasites with the help of phylogenetic

tree analysis method (UPGMA) (Nei and Kumar, 2000; Felsenstein, 2004 and Hall, 2011).

## MATERIALS AND METHODS

### Sample collection

The gastrointestinal tracts of goat from different region of Lucknow were collected and brought to the laboratory at Babasaheb Bhimrao Ambedkar University, Lucknow (Uttar Pradesh). Collected gastrointestinal tracts were dissected and thoroughly examined for presence of the parasites. The study was conducted in during 2017 depended on optical condition (temperature and humidity). June and July are hottest months (av. max. and mini. temp. of 29°C and 12°C temp., respectively) whereas December and January are the coldest month (av. max. and mini. temp. of 12°C and 4°C, respectively). Parasites were preserved in 70% alcohol in -20 deep freezer for further studies.

### Morphological identification

Gastrointestinal parasites were identified under compound microscope (10X and 40X) on the basis of morphology (Soulsby, 1982 ).

### Molecular analysis

Total no. of 50 parasites was crushed with ddh2o. Sample was digested by Lysis buffer. DNA extraction was done by P: C: I (25:24:1) method (Harris *et al*, 2001) according to the standard instructions. Quantification of isolated DNA from desired sample was done by spectrophotometer and gel electrophoresis unit (0.8.g agarose powder). Reported Primer was collected by Bandyopadhyay *et al* (2009) Forward-CATTGCAACATGCACTATG, Reverse-ACAGTTGTCATACAGGCCCC. PCR (Polymerase chain reaction) product was amplified with the help internal transcribed spacer 2 (ITS2) gene of the 3 nematode parasitic genome. rDNA gene was amplified for identification of intestinal *Oesophagostomum* species. The final 25µl reaction volume was set up using 10 µl TaqPCR Master mix, 2.0 µl of forward primer, 2.0 µl reverse primer, 8.0 µl DNA, 3.0 mili-Q water. PCR reagents were used for purpose of optimization. PCR set up was based on reaction, denaturation (94°C-95°C for maximum 1min), optimal temperature of annealing (54°C) extension (72°C for 10 mint) and 4°C cooling temperature followed by 35 cycles. DNA sample were amplified by the given primer and run on the 2% agarose gel electrophoresis alongwith DNA ladder. The DNA product band migration and ampflicons size was examined by UV transillumination technique. The PCR product was send for sequencing. After sequencing, sequence of particular species specific was performed and aligned for knowing homology by BLAST (Basic Local Alignment Search Tool, NCBI) multiple alignments, Clustal W (Thompson *et al*, 1997). Along with phylogenetic analysis was plotted by UPGMA method (MEGA 7.0 software) (Tamura *et al*, 2011).

## RESULTS

The morphological results of female parasites (av. length 1.2-24.5 mm and width 0.45 to 0.58mm) were larger than the male (av. length 1.5-03 cm and width 12.0 to 15.6mm) parasites. Color of parasites was observed opaque white. *Oesophagostomum* spp. contains a developed, multi-nucleate digestive tract as well as a

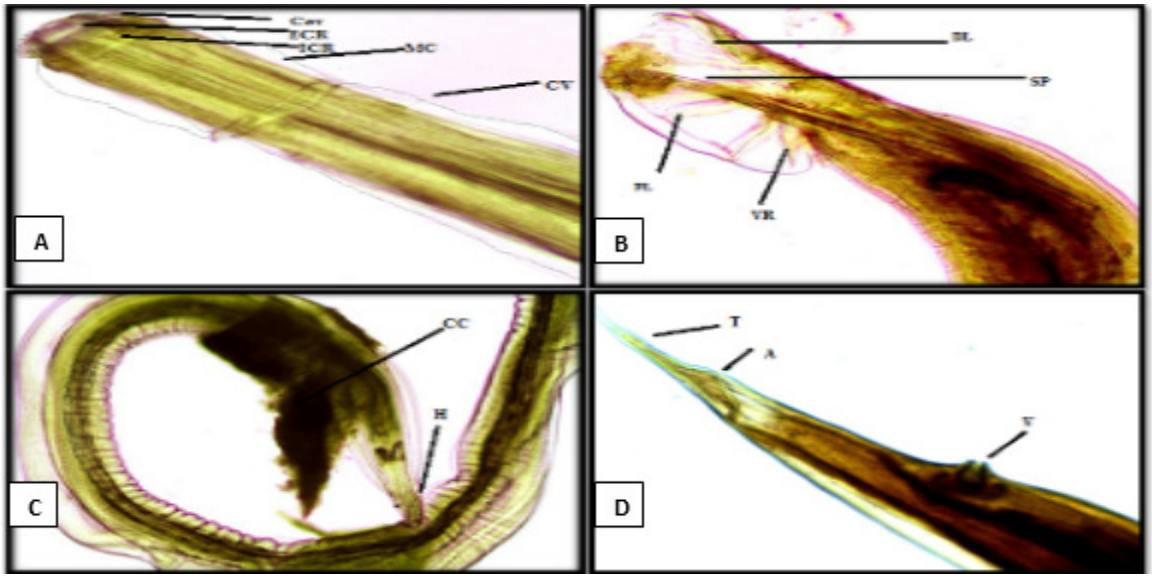


**Fig. 1 :** (A)- Anterior end of *Oesophagustomum* species (Cephalic vesicle (CeV), Internal Corona Radiata (ICR), External Corona Radiata (ECR), (B)- Posterior part of male (Dorsal lob (DL), (C)- Anterior part of female- Capulatory Cement (CC) (D)- Posterior part of female Vulva (V).

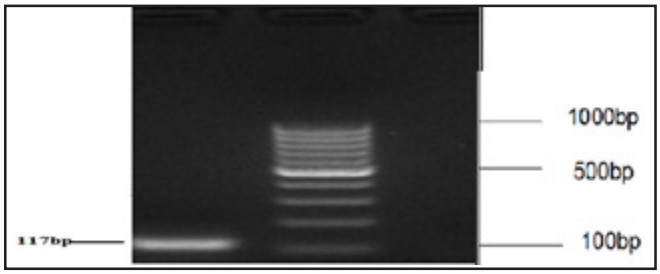


**Fig. 2 :** PCR purified product size 117bp loaded with 1 kb DNA ladder. 2% agarose gel showing Amplified DNA of *Oesophagostomum* species (OES3.1).

depends on microscopic technique, enable better understanding of parasites structure by morphologically (Zajac and Conby, 2006). Mouth cavity (MC) of parasites was interrupted at anterior region and mouth capsule (MC) was surrounded by 8 concentric rings. Cephalic papillae have developed on the exterior end (CP) and capulatory cement (CC) found in the anterior part of female. Male parasites have one specific character, bursa with a pair of lateral lobe, which was suited on the posterior part, similar studies have been reported by Yadav

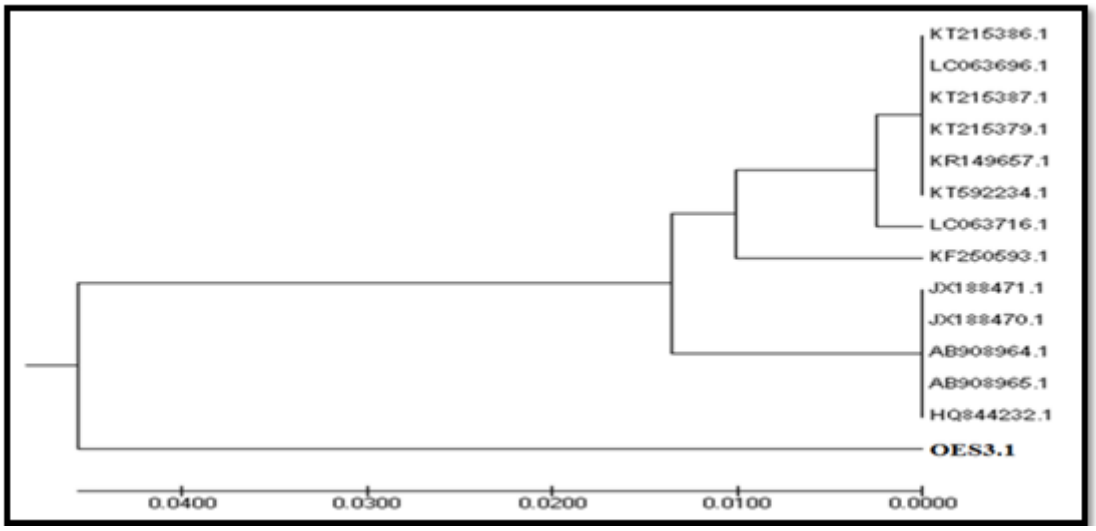


**Fig. 3 :** Phylogeny tree analysis was constructed by using UPGMA method. Accession no. for each sequence is given on the tree. The tree has been represented homology and evolutionary distances with the *Oesophagostomum columbianum* (OES3.1).

reproductive system. Males can be distinguished by their bell-like copulatory bursa, located in the tail and their paired rod like spicules. The mouth is bounded by oral papillae and cylindrical buccal capsule, as depicted above, is surrounded by a protective "external leaf crown" that is called the corona radiata. The eggs are indistinguishable fr om hookworm eggs. They have a thin shell and also range in size from 50 and 100 microns.

This investigation revealed that the *Oesophagostomum* spp. by molecular technique (PCR, DNA sequencing and bioinformatics). The amplified PCR product range range of approximately 117bp (Fig. 2). The generated sequence was blasted (BLAST) in nucleotide and compared to different accession number, revealed 98% homology, identified with *Oesophagostomum columbianum* after bioinformatics (BLAST, Clastal W, Oligo analyzer and Mega 7.0 software) generating sequence (OES3.1). The phylogenetic relationships with various species of parasites was represented that *Oesophagostomum columbianum* is closely related to *O. asperum* and *O. venulosum*.

## DISCUSSION

Identification of gastrointestinal nematode parasites and Tandon (1992). Singh (2003) and Soulsby (1982) have also reported that occurrence of twenty one leaf shaped elements in external corona radiata (ECR) at the region of the head of the parasites. Some specific character like dorsal lobe, posterior Lobe, ventral Lobe and spicules (DL PL VR and SP) was found in the male parasites. The specific character of parasites (capulatory bursa and spicules) was showed that the permeable structure of this species (Duggal and Kaur, 2006).

Molecular techniques have been widely used for identification and diagnosis of parasitic and their diseases (Dahlgren *et al*, 2010). Ghai *et al* (2014) have reported that PCR technique are most sensitive than microscopy for analysis of *Oesophagostomum* species. Abbaszadegan *et al* (2007) reported that PCR is used as diagnostic methods and observed the genetic variability. rDNA sequences are most important molecular markers used for differentiation of parasites (Rosenthal *et al*, 2008 and Xiang *et al*, 2009). Bandyopadhyay *et al* (2009) reported similar study on the ITS-2 region used for identification of different species of *Oesphagostomum* (nodular worm) parasite (*O. columbianum* and *O. venulosum*,). Sweeny *et al* (2011) have reported the PCR

technique based study. Kimura,(1980) have also been that the phylogenetic tree of the ITS-2 sequences were constructed by UPGMA method in the MEGA7 program (Saitou, 1987; Chilton *et al*, 1995; Tamura *et al*, 2007 and 2011; Lin *et al*, 2011; Ota *et al*, 2015; Kumar *et al*, 2018).

## CONCLUSION

The present study was based on the morphologically identification and molecular characterization of *Oesophagostomum columbianum*. The findings of this study revealed the data are detection of *Oesophagostomum* species (117bp-OES3.1 length of band). ITS-2 region were using rDNA interspecific difference and showed genetic relationship. The online blasting (BLAST) revealed that the nucleotide sequenced has findings 98% homology with *Oesophagostomum columbianum* (OES3.1). The study provided the molecular evidence of parasitic infection in Lucknow region. Molecular method having rapid changes in progress of research field with in significant genetic diversity. This observation might have implication for further study epidemiology, diagnosis of parasitic infection and control program.

## REFERENCES


Abbaszadegan R M, Velayati A and Tavasoli A (2007) Rapid DNA Extraction Protocol from Stool, Suitable for Molecular Genetic Diagnosis of Colon Cancer. *Iran Biomed. J.* **11**(3), 203-208.

Ahmed M, Singh M, Bera A, Bandyopadhyay S and Bhattacharya D (2011) Molecular basis for identification of species/isolates of gastrointestinal nematode parasites. *Asian Pac. J. Trop. Med.* **4**(8), 589-593.

Anderson R C (2000) Nematode parasites of vertebrates: their development and transmission. CABI Publ, UK, 47-314.

Baron S and Wakelin D (1996) Helminths: pathogenesis and defenses. *Medical Microbiology.* 4th ed. Galveston,TX, USA.

Blotkamp J, Krepel H P, Kumar V, Baeta S, Van't Noordende J M and Polderman A M (1993) Observations on the morphology of adultsand larval stages of *Oesophagostomum* sp. iso-lated from man in northern Togo and Ghana. *J. Helminthol.* **67**, 49-61.

Chilton N B, Gasser R B and Beveridge I (1995) Differences in a ribosomal DNA sequence of morphologically indistinguishable species within the *Hypodontus macropi* complex (Nematoda: Strongyloidea). *Int. J. Parasitol*. **25**, 647–651.

Chitwood B G (1931) A comparative histologicalstudy of certain nematodes. *Zeitschrift fur Mor-phologic und Okologie der Tiere* **23**, 237-284.

Dahlgren S S and Gjerde B (2010) The red fox (Vulpesvulpes) and the arctic fox (*Vulpes lagopus*) are definitive hosts of Sarcocystisalces and Sarcocystishjorti from moose (Alcesalces). *Parasitol.* **137**, 1547-1557.

Dorris M, Viney M E and Blaxter M L (2002) Molecularphylogenetic analysis of the genus Strongyloide sandrelated nematodes. *Int. J. Parasitol.* **32**, 1507 – 1517.

Duggal C and Kaur H (2006) SEM studies on the copulatory apparatus of male *Oesophagostomum columbianum*. *Helminthologia* **43**(1), 3–5.

Felsenstein J and Felenstein J (2004) *Inferring phylogenies* (Vol. **2**, p. 664). Sunderland, MA: Sinauer associates.

Gasser R B and Newton S E (2000) Genomic and genetic research on bursate nematodes: significance, implications and prospects. *Int. J. Parasitol*. **30**(4), 509– 534.

Gasser R B, Chilton N B, Hoste H and Beveridge I (1993) Rapid sequencing of rDNA from single worms and eggs of parasitic helminthes. *Nucleic Acids Res.* **21**(10), 2525-2526.

Gasser R B, De Gruijter J M and Polderman A M (2006) Insights into the epidemiology and genetic make-up of Oesophagostomum bifurcum from human and non-human primates using moleculartools. *Parasitol.* **132**, 453–460.

Ghai R R, Chapman C A, Omeja P A, Davis T J and Goldberg T L (2014) Nodule worm infection in humans and wild primates in Uganda: cryptic species in a newly identified region of human transmission. *Trop. Dis*. **8**, 26-41.

Goodey T (1924) The anatomy of *Oesophagostomum dentatum* (Rud.) a nematode parasite of the pig, with observations on the structure and biology of the free-living larvae. *J. Helminthol.* **2**, 1-14.

Hall B G (2011) Phylogenetic trees made easy: A how to manual (No. 576.88 H174p) Sinauer.

Harris T H, Szalanski A L and Powers T O (2001) *Molecular Identification of Nematodes Manual*. Publication at: https://www.researchgate.net/publication/236783621.

Hartwich G (1994) Strongylida: Strongyloideaund Ancylostomatoidea. In Dahl F: Die Tierwelt Deutschlands, 68. Teil. G. Fischer, Jena. 157 pp.

Holmes P H (1985) Pathogenesis of trichostrongylosis.*Vet Parasitol.* **18**, 89–101.

Holmes P H (1993) Interactions between parasites and animal nutrition: the veterinary consequences. *The Proc. Nutrition Soc.* **52** (1), 113-120.

Hoste H, Chartier C and Le Frileux Y (2002) Control of gastrointestinal parasitism with nematodes in dairy goats by treating the host category at risk. *Vet. Res.* **33**(5), 531-545.

Karp A, Seberg O and Buiatti M (1996) Molecular techniques in the assessment of botanical diversity. *Ann. Bot.* **78**, 143-149.

Kumsa B, Tadesse T, Sori T, Dugum R and Hussen B (2011) Helminths of sheep and goats in Central Oromia (Ethiopia) during the dry season. *J. Anim. Vet. Adv*. **10**, 1845-1849.

Lin Q, Li H M, Gao M, Wang X Y, Ren W X, Cong M M, Tan X C, Chen C X, Yu S K and Zhao G H (2012) Characterization of Baylisascaris schroederi from Qinling subspecies of giant panda in China by the first internal transcribed spacer (ITS-1) of nuclear ribosomal DNA. *Parasitol. Res*. **110**, 1297-1303.

Marigo J, Thompson C C, Santos C P and In Þiguez A M (2011) The Synthesium Brachycladiidae Odhner, 1905 (Digenea) association with hosts based on nuclear and mitochondrial genes. *Parasitol. Int.* **60**, 530 – 533.

Nei M and Kumar S (2000) *Molecular evolution and phylogenetics*. New York Oxford University Press.

Odoi A, Gathuma J M, Gachuiri C K and Omore A (2007) Risk factors of gastrointestinal nematode parasite infections in small ruminants kept in smallholder mixed farms in Kenya. *BMC Vet.*

*Res.* **3**, 1746-6148.

Ota N, Hasegawa H, McLennan M R, Kooriyama T, Sato H, Pebsworth P A and Huffman M A (2015) Molecular identification of *Oesophagostomum* spp. from 'village' chimpanzees in Uganda and their phylogenetic relationship with those of other primates. *Royal Soc. Open Sci.* **2**, 150-471.

Punja Z, De Boer S H and Sanfacon H (2008) *Biotechnology and plant disease management*. pp.195.

Rahman M H, Soliman K N and Shaikh H (1975) *Proceedings of Pakistan Scientific Conference*. University of Peahwar, 17.

Singh K S (2003) *Veterinary Helminthology*. New Delhi, ICAR. pp. 354–366.

Skryabin K I, Shikhobalova N P, Schulz R S, Popova T I, Boev S N and Delyamure S L (1992) *Key to Parasitic Nematodes*. **3**, 912 pp.

Soulsby E J L (1982) Helminths, arthropods and protozoa of domesticated animals. Bailliere Tindall, London **7**, 186–191.

Soulsby E J L (1986) *Helminthes, Arthropods and Protozoa of Domesticated Animals*. 7th edition, Bailliere Tindall, London. 809 pp.

Stevenson L A, Chilton N B and Gasser R B (1995) Differentiation of *Haemonchus placei* from *H. contortus* (Nematoda: Trichostrongylidae) by the ribosomal DNA second internal transcribed spacer. *Int. J. Parasitol.* **25**, 483-488.https://doi.org/10.1016/0020-7519(94)00156-I

Sweeny J P A, Robertson I D, Ryan U M, Jacobson C and Woodgate R G (2011) Comparison of molecular and McMaster techniques to confirm the presence of naturally acquired strongylid nematode infections in sheep. *Mol. Biochem. Parasitol.* **180**, 62-67.

Tamura K, Dudley J, Nei M and Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol*. **24**, 1596-1599.

Tamura K, Dudley J, Nei M and Kumar S (2011) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0., *Mol. Biol. Evol*. **24**, 1596-1599.

Tan T K, panchad C, low L V, Lee L V, Ngui R, Sharma S K R and Lim A L Y (2014) Co-infection of Haemonchus contortus and *Trichostrongylus* spp among livestock in Malaysia as reveled by amplification and sequencing of internal transcribed spacer Ð DNA region. *MBC Veterinary Res.* **10**, 38.

Taylor M A, Coop R L and Wall R L (2007) *Veterinary Parasitology*. 3rd edition. Oxford: Blackwell Publishing.

Thompson J D, Gibson T J, Plewniak F, Jeanmougin F and Higgins D G (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876 – 4885.

Xiang Z, Chen X, Yang L, He Y, Jiang R, Rosenthal B M, Luan P, Attwood S W, Zuo Y, Zhang Y P and Yang Z (2009) Non-invasive methods for identifying oocysts of *Sarcocystis* spp. from definitive hosts. *Parasitol. Int.* **58**, 293-296.

Yadav A K and Tandon V (1992) Stereoscan studies of two species of the genus *Oesophagostomum* (Nematoda, Chabertiidae). *Acta Parasitol*. **37**(3), 135–137.

Zajac A M and Conboy G A (2006) Veterinary clinical parasitology 7th. *Canadian Vet. J*. **48**(2), 198.