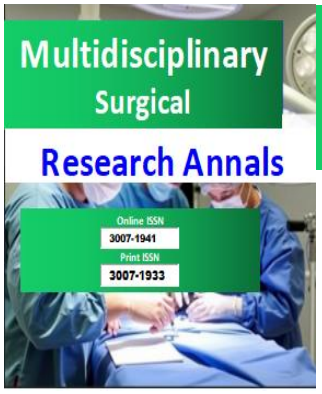

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Prevalence, Potential Risk Factor Analysis Of Haemonchusis And Its Effect On Blood Parameters In Sheep In District Karak

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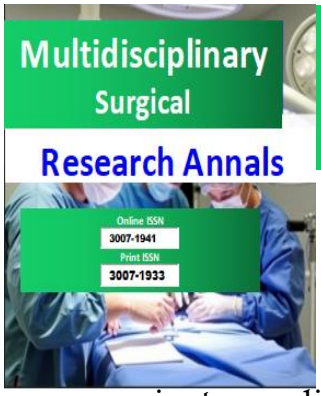
Abstract

Haemonchus is the most common infective disease in small ruminants caused by Haemonchus contortus, a blood sucking parasite causing anemia that may be fatal particularly to young animals. The current study was carried out to find out the prevalence, identification of associated potential risk factors and its effect on blood parameters in apparently healthy sheep in district Karak. During this study a total of 500 fecal samples were collected randomly using



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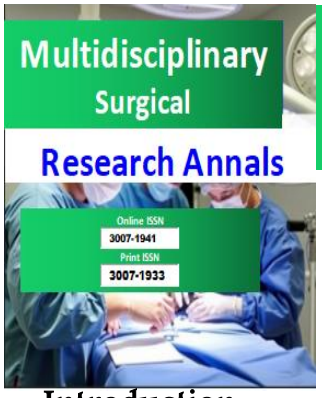
convenient sampling technique. Both blood and fecal samples were collected at one time in EDTA tubes and plastic bottles respectively and stored in refrigerator at 4°C degree centigrade till processing. During first week fecal and blood samples were collected and processed on 2nd week in the laboratory of Clinical Medicine and Pathology, College of Veterinary sciences, Abdul Wali Khan University Mardan. Out of 500 sheep samples, only 175 sheep were positive for this infection and the overall prevalence was 35% where the highest prevalence was observed in Tehsil Karak (70/500 (14%), followed by Banda Daoud Shah (55/500 (11%) while the lowest in That-e-Nasrati (50/500, 10%) and statistically highly significant difference (P< .01) was observed among different localities. The highest prevalence was recorded at the age of 1-6 months (42.28%) followed by 7-12 months (28%), 1-2 years (17.15%), while the lowest at the age of 2-3 years (12.57%) and statistically highly significant difference (P<0.04) was observed among different ages. Hematological analysis revealed marked decrease in hemoglobin contents, WBCs and blood platelets while other blood parameters were remained same.

Keywords: Haemonchus, Haemonchus contortus, Hematological parameters, Mardan, Platelets, Prevalence and Ruminants.



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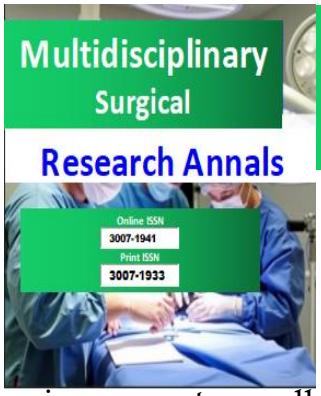
Introduction

Sheep are found throughout the world, are well adapted to various climates, and are used in all types of farming, but they are most prevalent in arid and semi-arid regions. They are grazing animals primarily. For meat and manure, they are especially domesticated. In addition to meat, they offer a wide variety of other goods, including hide, milk, skin, horns, the fiber wool, and medicine. The production of sheep meat is widespread worldwide. According to FAO data (2013) China held the majority with a 24% share. The next two biggest producers are Australia and New Zealand, with 8% and 5% of global production, respectively (Colby 2016). In sub-Saharan Africa, sheep are thought to supply up to 30% of the animal's flesh and 15% of the milk (Gizachew, et al., 2014). Sheep and goats are significant farming animals because they require less capital investment, have shorter production cycles, grow more quickly, and are more tolerant of their environment compared to cattle (Sissay et al., 2008). Sheep have evolved to adapt to a variety of environments, including the hot, arid pasturelands of the lowlands and the cool, alpine weather of the mountains (Hassen and Tesfaye, 2014). Due to their small bodies, low metabolic needs, and minimal maintenance requirements in semi-arid and arid regions, they also require less feed than cattle do (Bikila and Urge 2013). This makes it simple to



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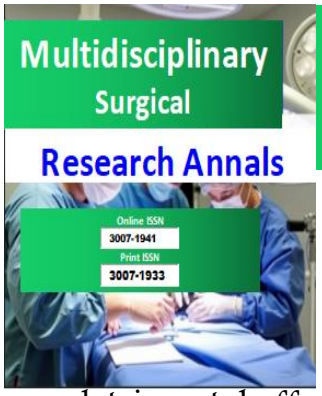
incorporate small ruminants into various farming systems. Sheep and goats are a major source of income for farmers and pastoralists in the subsistence sector (Hirpa and Abebe, 2008; Kenya et al., 2015). Additionally known as poor man cows. They also significantly contribute to the social and food security of rural populations who endure severe deprivation (Duguma et al., 2010).

Because of the hematophagous parasitic behavior of H. contortus, infection causes anemia, which may occasionally result in mortality of the infected livestock. According to Fetterer and Rhoads, a different pathogenetic mechanism for the disease involved the helminth producing a hemolytic factor that led to distinct changes in morphology on the surface of the sheep's erythrocytes. But the infection's primary impact is the sharp fall in the output of animals with infections, resulting in an overall decrease in young animals' growth and a decline in the milk output of lactating animals, and a decline in the manufacture of fiber (Fetterer and Rhoads, 1998; Fthenakis and Papadopoulos 2018). All of these ultimately cause farmers to suffer significant economic losses (Arsenopoulo, et al., 2021).

The growing demand of meat, milk, and wool can be affected by gastrointestinal helminths, which are a major cause of decreased productivity (Coop and Holmes, 2020). Additionally, digestive tract nematode infestation had



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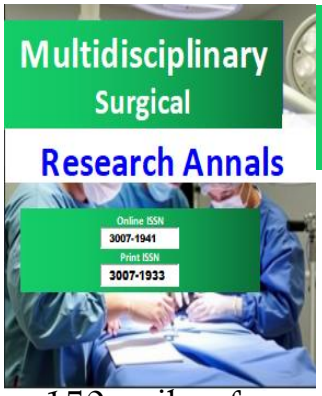
a detrimental effect on both the rate of wool growth and the mean daily gain of body weight (Niezen et al., 2018). In particular in tropical as well as subtropical regions, gastrointestinal nematodes are a major problem for sheep and cause significant losses (Mendoza et al., 2019). Giessen, Ramos, and others (2010) Sheep and goats raised in highly and wide production systems are particularly vulnerable to the effects of a wide variety of helminth endoparasites, which continue to be a significant productivity constraint. (Kenea et al., 2015). The acute illness and deaths brought on by these parasites, the premature slaughter, and the rejection within some parts during meat inspection are all blamed for the direct losses. (Abouzeid et al., 2010), (Sultan et al.,2016) both list the reduction of productive potential as one example of indirect losses. Other examples include slower growth rates, decreases in weight in young growing animals, and a later stage of slaughter stock.

Martials and Methodology

Karak is a district of Khyber Pakhtunkhwa in Pakistan is located between 70.40° and 70.30°N latitude and 32.48° and 33.23°E longitude. It is located 340 meters above sea level. The district's total population was 7,06,299 people, as reported in the 2017 census report (GOP 2007). On the Indus Highway N-55 (from Peshawar to Karachi), District Karak lies in Khyber Pakhtunkhwa (Pakistan),



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150 miles from Peshawar. Karak district is geographically arid with 330 millimeters of yearly rainfall. Commercial enterprise is restricted by the lack of water and some regions' arid environment.

Ethical Approval

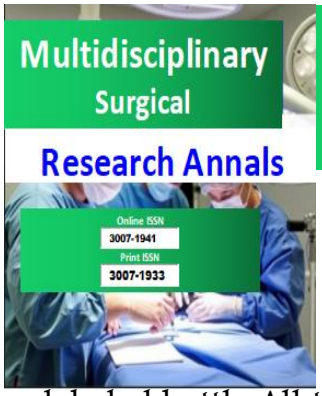
The Research Ethics Committee at the (CVS & AH) College of Veterinary Sciences and Animal Husbandry Abdul Wali Khan University Mardan, Pakistan, approved all the research and experimental protocols/procedures. All the samples were taken from the sheep and lambs in accordance with the recommendations and instructions of the Ethical committee and advanced laboratory procedure was adopted.

Sample Size

During this study a total of 500 fecal and blood samples were collected randomly from sheep . During 1st week samples were collected and stored in refrigerator while on 2nd week the samples were examined.

Sample Collection

During collection, 8-9 samples were collected randomly on each day. All the protection necessary such as gloves, disposable bottle, mask and white coat were adopted before collection of samples. The 10gm feces were directly collected from the rectum or freshly passed feces were also collected and stored in a



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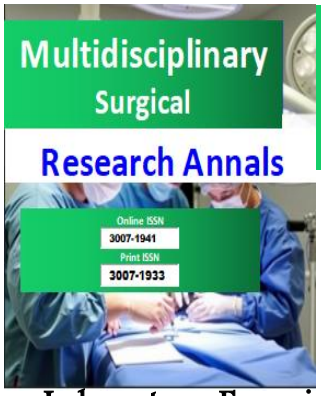
labeled bottle All the necessary information were enter n the questionnaire. The same process was repeated for all collected fecal samples. Necessary information was recorded clearly such as age, sex, name of owner, feeding behavior, date of collection and season of fecal samples collection was properly recorded. The current study was performed between February 2023 to June 2023 to calculate the prevalence of H. contortus and associated risk factors. All the samples of sheep were randomly collected from farms, pastures, and slaughterhouses and then checked for H. contortus egg. Using a saturated solution of sodium chloride, the flotation technique was applied to evaluate the fecal samples of sheep. The eggs gathered in normal saline and identified using Soulsby's criteria (1982). In order to show the significance of risk factors in the prevalence of Haemonchus infection, the sexes, ages, and physical conditions were taken into account in this study. The inclusion of the sexes, ages, and physical conditions in this study served to highlight the part played by host factors in the prevalence of Haemonchus infection. In order to include additional flocks in the study and highlight the seasonal variations in infection prevalence and intensity, data collection repeated each month.

Sample Preservation Technique

Fecal samples were preserved in 10% formalin.



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Laboratory Examination

The fecal samples were brought to laboratory of Clinical Medicine and Veterinary Parasitology, College of Animals Husbandry and Veterinary Sciences, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan.

Meteorological Data

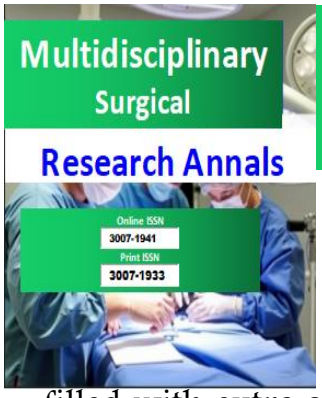
During collection the metrological data of district Karak was also recorded on daily basis such as temperature, humidity and rainfall etc.

Saline Wet Mount

Approximately 10 gram of fecal sample was taken and added 5 ml of saturated sodium chloride solution then the sample was grinded lightly with the help of patula. One drop of Iodine solution was placed on cover slip then the crushed fecal sample was placed on that iodine with the help of tooth pick and stirred it well then cover slip was placed on it (Soulsby, 1982).

Differential Flotation Technique

For finding nematode eggs, this method is frequently utilized. Their eggs may float in the flotation liquid because they are lighter and smaller. A fecal sample weighing around 2 grams was placed in a beaker with 10 ml of saturated sodium chloride solution. The material was then lightly ground with a spatula, and the fluid was filtered through a tea strainer. A 10 ml centrifuge tube was



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filled with extra sodium chloride solution after the filtrate solution was added, and the tube was run at 1,000 rpm for five minutes.

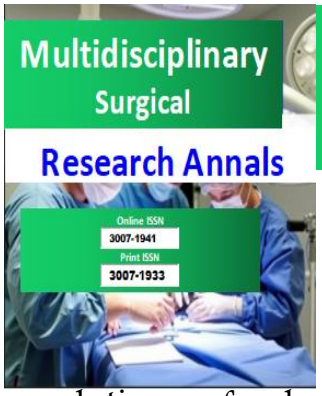
More concentrated sodium chloride was added after centrifugation to produce a convex surface at the tube's apex. A cover slip was then added, and after five minutes it was removed and put on a slide for inspection using 10X and 40X objectives. After that, one drop of methylene blue was added. Based on color, shape, and size (morphometry), parasite eggs were photographed and identified. (Souls by, 1982).

Sedimentation Technique

The nematode eggs were found by using a sedimentation method. After analyzing the flotation portion, saturated salt solution being softly removed from the test tube, the sediment portion was placed into a watch glass, and the mixture was gently mixed. For the second slide, one drop of the mixture was used to prepare it. With iodine wet mounts solution, the specimen was stained. In this manner, two slides from a single sample (one from floating and one from sedimentation) were created, and they were then examined under microscopes with 10X and 40X objectives to look for eggs (Souls by, 1982)

Fecal Qualitative Examination

Using zinc sulphate ($ZnSO_4.7H_2O$) with a specific gravity of 1.27 as a flotation



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solution, a fecal qualitative investigation was carried out with the use of the fecal flotation technique. [97]

Microscopy

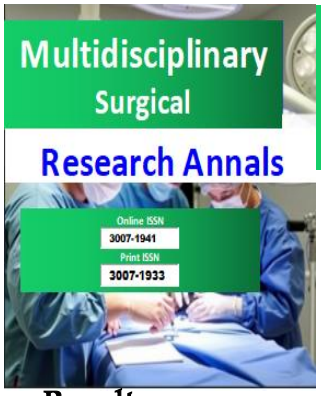
The samples were observed under microscope for identification in the laboratory of Clinical Medicine and Veterinary Pathology, College of Animals Husbandry and Veterinary Sciences, AWKUM.

Hematological Analysis Technique

The score for body condition calculated and classified as good, medium, and bad. The blood samples collected through syringes and added to EDTA containing tubes. The blood samples checked and analysis in the laboratory of Clinical Medicine and Veterinary Parasitology, College of Animals Husbandry and Veterinary Sciences, AWKUM.

Statistical Analysis

The data was entered in Microsoft Excel, which was utilized to analyze simple descriptive statistics. Using IBM-SPSS version 24.0, for descriptive statistics. The P-value of < 0.05 will be advised significant in the statistical analysis for the difference in the prevalence of H. contortus across risk variables, with the confidence level being retained at 95%.



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Results

Out of 500 (male 150 and 350 females) sheep samples only 175 sheep were positive for Haemonchus infection in various selected tehsils of district Karak where the overall prevalence was 35%.

During this study area wise prevalence was investigated where the highest prevalence (14%) was recorded in tehsil Karak, fallowed by Bonda Daoud Shah (11%) while the lowest in the tehsik That-e-Nasrati (10%) and statistically highly significant difference was observed among different localities.

S.No	Region	Male	Female	Prevalence(%)	P.Value
1	Banda Daoud Shah	16	39	11%	
2	That-e-Nasrati	12	38	10%	
3	Karak	17	53	14%	
4	SUM	45	130	35%	
5	AVERAGE	15	43.33333	12%	
6	MEDIAN	16	39	11%	
7	CONFIDENCE	95.00%	95.00%	95.00%	

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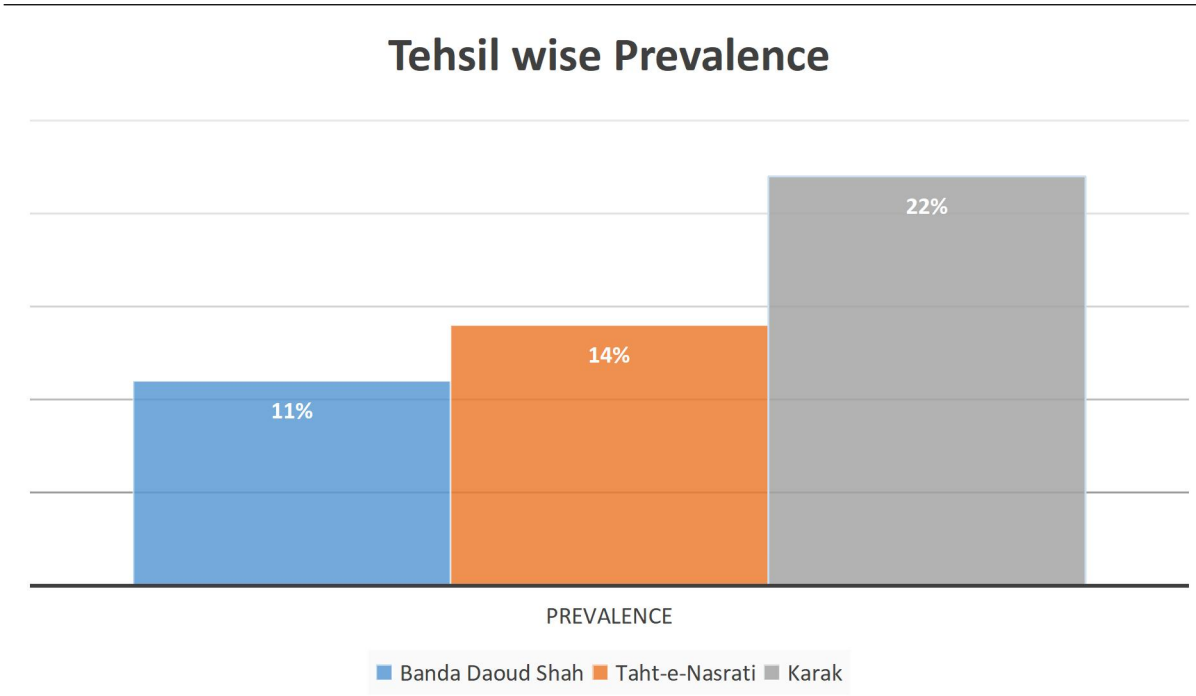

During current study it was predicted that females were more prevalent to Haemonchus compare to males due to their grazing, pregnancy, location, weakness, immunity and parental care which makes their exposure to various environment. The graph is shown below;

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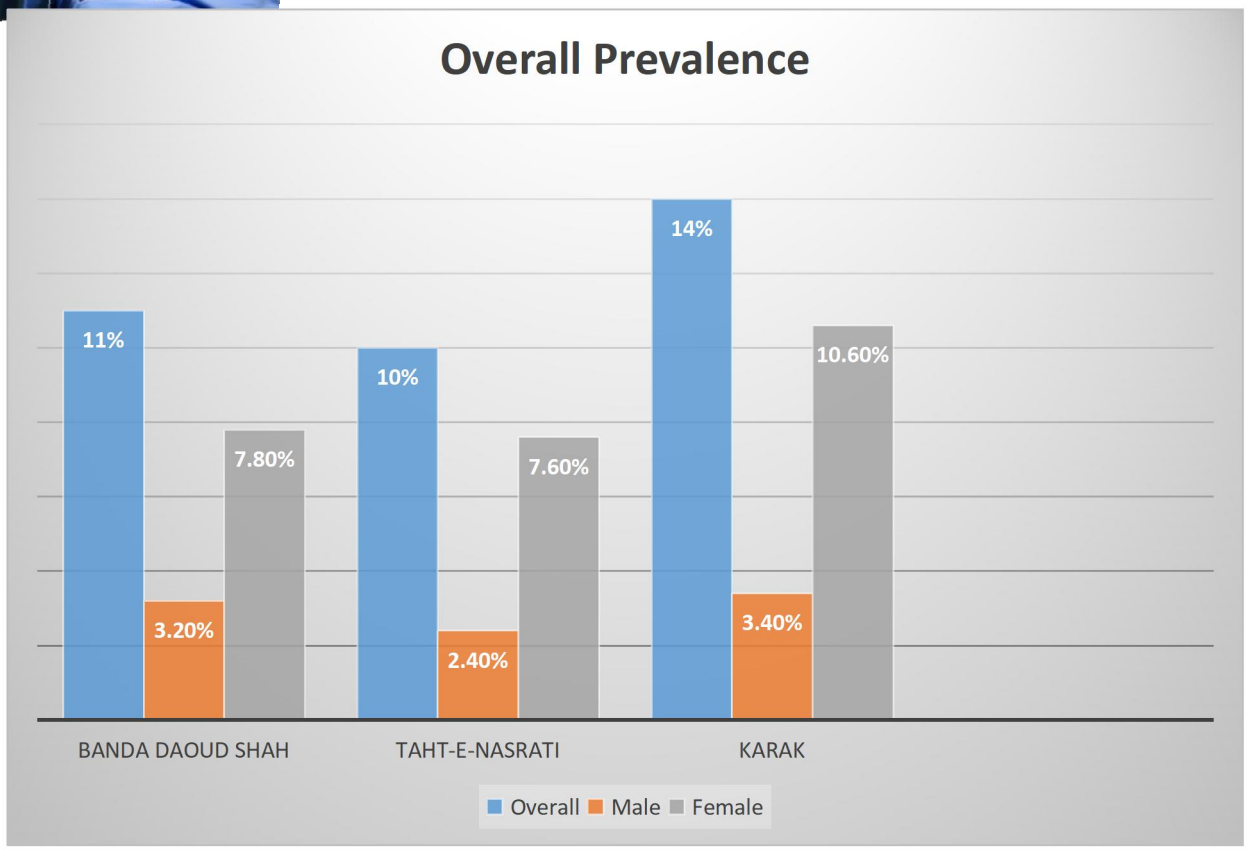

Figure 4.2 Sex wise Prevalence

During this study age wise prevalence was also investigated where the highest prevalence (42.28%) was recorded at the age of 1-6 months, followed by 7-12 months (28%), 1-2 years (17.4%) while the lowest (12.57%) at the age of 2-3 years. Statistacally highly signficant difference (P<0.0093) was recorded.

S.No	Age	Prevalence	Percentage	P.Value
1	1-6 Months	74/175	42.28%	
2	7-12 Months	49/175	28%	
3	1-2 Years	30/175	17.14%	

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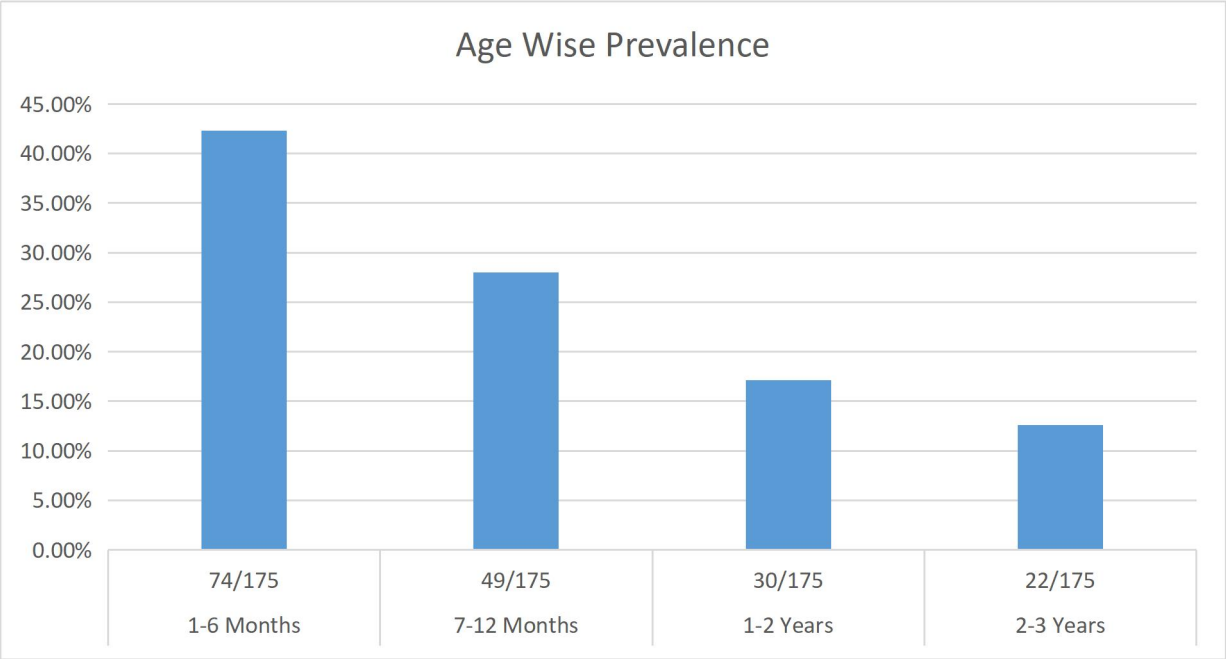
4	2-3 Years	22/175	12.57%	0.00938



Blood samples were analyzed through automated hematology analyzer .Blood samples were collected from positive as well as negative cases. Out of 100 samples, 50 from infected animals while 50 from healthy sheep were selected and examined. The positive cases parameter is given below.

Blood components	Infected sheep	Healthy sheep	Units
Haemoglobin	3.4-9.7	9-15	gm/dl
Total WBC Count	3,200 - 8,400	5000-15000	µL
Polymorphs	51-71	40 - 70%	%
Lymphocytes	24-43	20-40%	%

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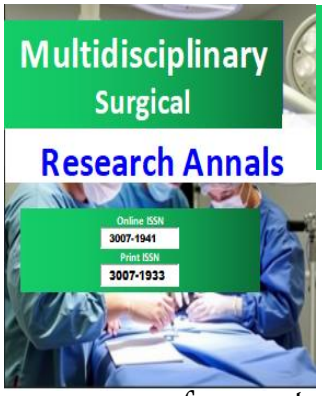
Monocytes	02-05	2-6%	%
Eosinophils	01-05	1-6%	%
Platelets Count	34,000-94,000	200000-500000	µL

Table 4.4 Comparative analysis of blood composition in infected and healthy sheep with Haemonchus infection. During this study the blood parameter were studied in detail. As a result, there was much decrease in Hb, WBCS and Monocytes. The Hb level in infected sheep was ranged from 3.4- 9.7 mg/dl while the Hb level in healthy sheep was 9-15 mg/dl WBCS, Monocytes. There was no significant change in count of Polymers, Lymphocytes and Eosinophils while much increased in Platelets.

Discussion

This research work was carried out on sheep of various localities in district Karak. During fecal sample flotation and hematological analysis it was determined that overall prevalence of sheep is 35 %. In comparison of 3 tehsils, tehsil karak showed highest prevalence compared to other. The highest prevalence was observed in female sheep due to their exposure to various environment. While male have limited activities and strong immune system make them less prevalent. Highest prevalence ratio is found in tehsil karak i.e. 10.6 % while lowest in Taht-e-Nasrati i.e., 7.6%(Tefera et al., 2009). Eight



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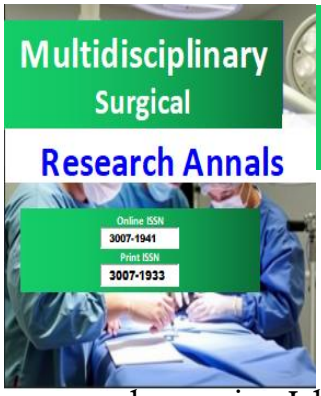
genera of nematodes were found in sheep, with Haemonchus sp. having the highest prevalence rate of 67.5%, following by Trichostrongylus sp. (48.8 %), Trichuris sp. (46.1%), Oesophagostomum sp. (48.8 %), Bunostomum sp. (30.3 %), and Ostertagia sp. (25.6%). The prevalence is high in comparison to recent study because of ecological and seasonal variations. As in Ethiopia there is no proper checking and care of sheep farms. Due to maintenance of hygienic condition in Karak environment the prevalence is lowest comparatively. Secondly the pasture in Ethiopia is wild one while in karak it is domestic one which are properly sprayed by pesticides.

(Brik, et al., 2019) Conducted a study in Morocco and observed 23.92% prevalence while we observed 35% overall prevalence this increase may be due to health status of sheep, breeds. Immune status and area to area variation, climatic changes and level of contamination.

(Boukhari, et al., 2016) conducted a study in Khartoum to determine the prevalence of H.contortus eggs in sheep feces, Their overall prevalence was 12.1% which is much lower than our study while in our study the prevalence is 35% this difference may be due to health status of sheep, breeds. Immnue status and area to area variaton,climatic changes and level of contamination.

(Asif et al., 2008) also conducted study in Pakistan and observed 72%



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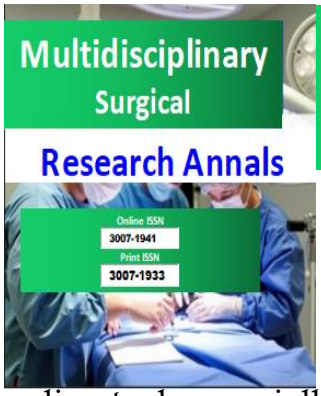
prevalence in Islamabad while we observed 35% during our study. The difference may be due to area to area variation, level of infection, humidity, breeding, season and stage of pregnancy.

Hemonchusis is a very common disease in small ruminants livestock caused by Haemonchus contortus, a blood-sucking parasite that can be fatal, especially to young animals. (Ali asghar Tehrani et al., (2012). carried out studies on histo-pathological study of haemonchus contortus in herrik sheep abomasum in Iran. The goal of the current investigation was to detect the frequency of Haemonchus contortus in sheep that had been slaughtered at the Urmia abattoir in Iran's north-western region. Between July 2010 and July 2011, 2421 animals were killed and examined at the Urmia abattoir. 225 out of 2421 sheep tested positive for Haemonchus contortus, and the infection rate was 9.3%. In sheep, Haemonchus contortus was present in 33.08% (76/229) males and 66.22% (149/225) females, respectively. In comparison to male (33.08%), females showed a considerably (P0.05) higher prevalence (66.22%).

Conclusion

The current research showed that Haemonchusis is the most prevalent and common infective disease in small ruminants caused by Haemonchus contortus in district karak sheep population. The disease cause serious threat to health in



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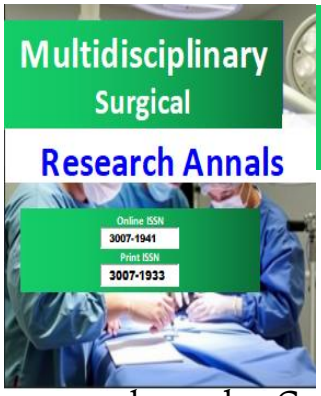
livestock especially sheep as it a blood sucking parasite causing anaemia and decrease blood cells and proteins number leading to mortility of young animals. The current study is carried out to find out the prevalence, potential risk factors and its effect on blood parameters in apparently healthy sheep.

Overall frequency of sheep infections with H. contortus is a major health issue, haemonchusis causes a reduction in productivity because of its high morbidity, mortality, and expense of treatment and control methods. The parasite may be destroyed by the harsh winters and scorching summers that account for the variation in occurrence. variations in haemonchusis based on the gender of the sheep. Due to reproductive stress and weakened immune systems, females are more vulnerable to parasitism, and is thought to be a decisive factor influencing the occurrence of haemonchusis.

The animals' low resistance to infection stems from their lack of prior exposure to nematode infections. As a result of increased exposure, the host animals' immune systems become more specialised in combating these parasites and develop age-related resistance. The reason for this difference in the infection rate is that animals with poor physical health have weakened immune systems against parasites. This high prevalence might be due to lack of awareness about the disease or due to improper checking status of sheep



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prevalence by Government veterinarian. There should be a proper awareness program or seminars to educate the local farmers regard this disease. The worldwide outbreak of haemonchusis is primarily related to waterborne and foodborne contamination. To control the spread of this enteric protozoan, a preventive education programme should be put in place. Unsanitary conditions for the care of humans and animals should be avoided, and hygienic procedures should be used wherever possible. Concentrating on cleaning contaminated surfaces and areas with appropriate disinfectants, using chlorinated water, and applying proper wastewater treatment can prevent the infection from spreading and transmitting to livestock.

Conflict of Interest

The authors do not have any conflict of interest to declare

Consent for Publication

Written informed consent was taken from the patient for case report and publication. None of personal information will be disclosed in the final publication.

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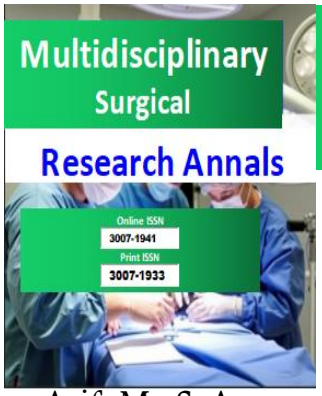

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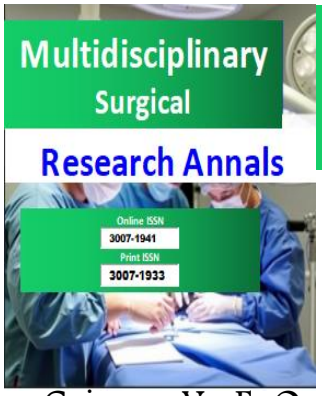

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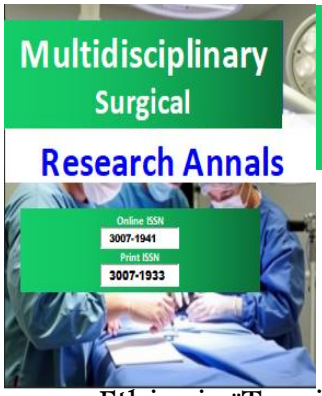

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