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Research Article Prevalence of protozoan parasites in gastrointestinal tract of Goats

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ABSTRACT

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Parasites Entamoeba Gastrointestinal Protozoan Qualitative techniques Protozoan parasites cause severe diseases in livestock including buffaloes. sheep, goats and human either by ingestion of contaminated meat or faeces containing oocysts, and also from drinking milk of infected cattle. In present study prevalence of gastrointestinal protozoan parasites were examined in goats. A total of 100 faecal samples were obtained from Lahore Meat Processing Unit (LMPC) to detect the presence of protozoan parasites like Entamoeba histolytica, Neospora species, Eimeria, Balantidium coli, Giardia and Cryptosporidium. Qualitative techniques (direct smear, floatation and centrifugation techniques) were used to identify parasites. Data analysis revealed that overall prevalence of protozoan in faecal samples was 56%. The percentage prevalence of Eimeria, Entamoeba, Balantidium and Neospora species, was 31, 13, 7 and 5%, respectively. Giardia and Cryptosporidium were not observed. Detailed analysis revealed that Eimeria species were observed. In which Eimeria ahasta had highest prevalence (20%) followed by Eimeria intricate (8%) and Eimeria arloingi (3%). Gender-wise comparison revealed that 24% male and 32% female goats were infected with protozoan. Challenges are there by the wide prevalence of protozoan so there is the need to bring awareness in public for control strategies to reduce parasitic load.

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Introduction

Rearing of goat has importance in global animal husbandry as goat provides milk and meat to humanities (Kagan et al. 2011). Animal husbandry is playing vital role in socio-economic growth in rural along with urban localities (Rafi et al. 2021). Ruminants are suffering with large variety of parasitic infection (Nouri et al. 2022). Parasites are causing heavy lose i.e., reduce the profitability of livestock industry up to 15% (approximately 19.7 million per year) as lowering the productivity of affected animals, improper growth and even lead to death (Guel 2007). Severe effects produce mortality and reproductive failure rate increases through parasites in domestic animals (Northrop-Clewes and Shaw 2000; Ghimire and Bhattarai 2019). Different protozoan parasites of wild animals at zoological gardens of the world, like oocysts of Entamoeba, Sarcocysts, Giardia, Cryptosporidium and sporozoite of Babesia have been recognized (Radhy et al. 2013). Various researches have shown that parasites cause public health infection like Balantidium coli and Entamoeba spp. as these are the source of zoonotic transmission (Elmadawy and Diab 2017). Several researchers observed that death rate produced by Entamoeba spp. was higher than the number of deaths caused from

schistosomiasis as well as malaria (Sebba et al. 2021). The domestic goat was considered as the earliest known animals, which is also well-known as poor man's cow (Guel 2007).

A study was conducted with the aim to determine the route of transmission of parasite (protozoan, helminths and disease causing parasite) in different cattle (Sultan et al. 2016; Elmadawy and Diab 2017; Mohamaden et al. 2018). The *Coccidia* (commonly spread by *Eimeria*) and *Cryptosporidium* (parasitic diseases) have been shown to cause worldwide economic lose in domestic animals (Wang et al. 2010; Khezri and Khezri 2013; Gadelhaq et al. 2015; Majeed et al. 2015). Australian scientist determined that dog is the definitive host of *Neospora* (Guel 2007). Dog act as definitive host for the dispersal of oocysts of *Neospora* through the ingestion of faeces by the intermediate host, such as cattle. It may be congenital type transmission i.e. mother to offspring.

Thirteen *Eimeria* spp. have been reported from the goats that destroy the villous epithelium and mucosa of host's intestinal cells leading to anemia and poor absorption of nutrients. *Eimeria christenseni, Eimeria ovinoidalis* and *Eimeria arloingi* have shown to be highly pathogenic in kids (Wang et al. 2010; Kagan et

al. 2011). This study is therefore performed with the aim to determine the percentage prevalence of gastrointestinal protozoan parasites along with various species of *Eimeria* in faecal samples of goat by using direct smear, flotation and centrifugation methods. The purpose of this study is to find the prevalence of protozoan in intestinal tract of goat as well as to reduce the threats by good hygiene conditions of housing and ventilation, depopulation of protozoa rearing points, limit the transmission through grazing to avoid fecal contamination and risk factors associated with protozoan infection.

Materials and Methods

Study design

Lahore Meat Processing Unit (LMPC) is a Government project under the Punjab Agriculture and Meat Company (PAMCO). It is now located at 18 km Multan Road New Baker Mandi Shahpur Kanjran Lahore. LMPC was inaugurated outside the city in 2011. Feacal samples were collected from September 2013 to March 2014 from the gastrointestinal tract of goat slaughtered in the slaughter house (LMPC). The fresh feacal samples from the rectum of male (n=50) and female (n=50) goats were collected in polythene zipper bags labeled for the parasitological examinations and preserved in refrigerator. The samples were transported to Lahore College for Women University laboratory for further observation. These polythene bags were airtight to prevent the rate of development and hatching of eggs.

Gross Examination

Before microscopic examination, gross examination was performed to describe the physical features of faeces, in parasite (protozoan) samples. Observation of any disorder in the feacal samples suggested that there may be incidence of any parasite. Age of faeces, consistency, color, odor, presence of blood and adult trophozoites parasite were observed in gross examination.

Faecal samples were observed by microscopic examination in smear for the purpose to observe the protozoan cysts. Various techniques like direct method, flotation method and sedimentation method were applied to detect the protozoan in faecal samples (as same techniques were applied in a research work conducted by Ruhoollah et al. 2021).

With the help of direct smear method cysts can be identified according to their shape, size and nuclei arrangements. In fresh feacal samples mobility of parasite can be seen through microscope because they can be observed by direct smear method as it is quick and simple technique.

It is commonly known as Fecalyzer or egg flotation as well as feacal floats. It is performed under the sodium nitrate flotation technique. It has worth after direct smear technique. Magnesium sulfate, sugar, zinc sulfate and sodium nitrate are the commonly known flotation fluids (Azrul et al. 2017).

The most appropriate technique (centrifugation) other than the direct saline and flotation method was performed under the principal of density gradient centrifugation. It requires short time interval than the other techniques. On heavier objects, a greater force is needed when flotation is applied in a centrifuge machine (Das et al. 2017; Hassan et al. 2019).

Data analysis

The Chi-square test (Hassan et al. 2019; Rafi et al. 2021) was applied to find out the relationship among several categories like percentage value of parasites in fresh faecal samples of goats. P-values <0.5 was considered as significant. The data was analyzed by using Minitab 16. By using Microsoft Excel 2007, results were expressed in graphical form.

Results and Discussion

The fresh faecal samples were collected a day before examination and then samples were kept in refrigerator to cease the growth of oocyst and next day the samples were taken for analysis in research laboratory at LCWU as early as possible in the morning. The infective stages of protozoan parasites were observed in freshly collected faecal samples. Therefore, fresh faecal samples of goats were used. Solid or watery consistency of fresh faecal sample indicated constipation and diarrhea respectively. Usual smell of faeces was noticed. Moreover, foul smell of faecal samples was also observed in some faecal samples. Color variations like green, brownish green and brown were observed in samples. In faecal samples gray color was not observed that could be due to the abundance of fat in liver. The results showed that no blood and adult parasite were found in fresh faecal samples of goats collected from Lahore Meat Processing Unit (LMPC). It was studied that gastrointestinal parasite were present in ruminants in Malaysia as well as at global level (Hao et al. 2018; Yusof and Isa 2016). In another research work it was observed that gastrointestinal infection parasites (GIPs) had high prevalence in South Africa and GIPs had possible effects of age, breed, sex and season (Mpofu et al. 2022). Literature review has revealed that *Eimeria* protozoan can survive for a long-time in infected goats and causes anemia, electrolyte loss and poor absorption (Wang et al. 2010; Cavalcante et al. 2012; Verma et al. 2018). Higher the incidence of parasitic infections in animals by grazing system lowers productivity, leading to economic losses. Entamoeba histolytica makes disturbance in gastrointestinal tract of cattle (Saritha et al. 2015). In tropical areas the potential of sheep and goats have been adversely affected by gastrointestinal parasites, which lead to countless effect even deaths (Soliman and Zalat 2003).

In different studies protozoa infection (Giardia, Eimeria and Cryptosporidium) was observed in small grazing animals especially in lambs, kids and cattle that produce important clinical disease which cause death in severe infection (Lianou et al. 2022; Wuthijaree et al. 2022). By microscopic examination, it was observed that Eimeria has highest percentage prevalence (31%) followed by Entamoeba histolytica, Balantidium coli and Neospora spp. with percentage prevalence of 13, 7 and 5% respectively. Statistical analysis revealed that faecal parasites had highly significant relationship with each other. Giardia is considered to be one of the leading causative agents of diarrhea. Goats grazed in natural pastures got infection with cysts of Giardia. The transmission of this infection is due to the ingestion of faeces or

contaminated water or food (Adam 2001; Windsor et al. 2018). Entamoeba histolytica was proved to be pathogenic microorganism for both humans and animals. Cryptosporidiosis mainly caused by *Cryptosporidium parvum* that is primarily the disease of lambs and kids. Fluid loss was observed as one of the most commonly known pathological effects of *Eimeria* and *Cryptosporidium* species in ruminants. Scientific research was performed for prevalence of *Eimeria*, *Giardia* and *Cryptosporidium* in small ruminants (Lianou et al. 2022).

Direct smear method analysis of data revealed that infection with oocysts of Eimeria and Entamoeba histolytica was 28 and 11% in goats. Neosporaspp. and Balantidium coli oocysts identified by direct smear method was 1% (Fig. 3). Analysis for the oocysts of Cryptosporidium and Giardia were made but they were not found in any sample. Oocysts of Eimeria ahasta had highest percentage (19%). The prevalence of Oocysts of Eimeria intricate and Eimeria arloingi were observed with 8% and 1%. Eimeria parva and Eimeria ovinidalis were not observed (Badran et al. 2012). Centrifugation method is used for more precise results in a very short time period by applying centrifugation i.e a force on floating liquid. Direct smear method was used to examine the faecal parasites of goats. Eimeria was found with 28% prevalence in all faecal samples. Examination of sample revealed that *Entamoeba histolytica* had 11% prevalence. The Balantidium coli and Neospora spp., when analyzed by direct smear method had percentage prevalence of 6% and 1% respectively. Giardia and Cryptosporidium were not observed by direct smear method. The results of present study were in agreement with findings in Cairo goats (El-Manyawe et al. 2010) that Eimeria had highest prevalence among other protozoans with 73% prevalence rate when examined by direct smear while Cryptosporidium and Giardia had low prevalence percentage. In another study it was determined that 7.1% goats suffered with balantidiasis infection (Elmadawy and Diab 2017). However, another research (Verma et al. 2018) showed high level of coccidian infection and Eimeria was not present in pure situation while it may present in combined form with other GIP (gastrointestinal parasite).

Analysis of data examined by flotation technique showed that there was 31% prevalence of Eimeria (Fig. 1) in faecal samples. It was revealed that Entamoeba histolytica, Balantidium coli and Neospora spp. (Fig. 2) had parasitological prevalence of 13, 6 and 5% respectively in faeces of goats (Fig. 3). The eggs of Cryptosporidium and Giardia were not observed in any sample. Eimeria was also identified with different species by this method. Oocysts of Eimeria ahasta were identified with high percentage prevalence of 19% while Eimeria intricate (8%) and Eimeria arloingiwere (4%) was also found in faecal samples of goats. It was also observed that oocysts of Eimeri aparva and Eimeria ovinidalis had no prevalence. During analysis of protozoan in faecal samples of goats by direct smear technique, it was observed that *Eimeria* had prevalence with its different species like Eimeria ahasta, Eimeria intricate, Eimeria arloingi, Eimeria parva and Eimeri aovinidalis. Eimeria ahasta had high prevalence (19%) while Eimeria intricate had 8% and Eimeria arloingi was observed in 1% in faecal

samples of goats while *Eimeria parva* and *Eimeria ovinidalis* had no prevalence in goats. Similarly, the study on protozoan parasites through direct smear method have revealed that, *Eimeria ahasta* had 53% prevalence followed by *Eimeria arloingi* (35.71%) (Ibrahim 2012), whereas *Eimeria parva* and *Eimeria ovinidalis* was not found in this study.

For the observation of protozoan in faecal samples of goat through flotation method suggested that Entamoeba histolytica and Eimeria were clearly observed with high prevalence with 13 and 31% respectively, whereas, Giardia and Crptosporidium were not observed. Balantidium coli and Neospora spp. had low prevalence rate with 6 and 5% respectively. Similar research work was directed previously (Sangvaranond et al. 2010; Sutaaker et al. 2017) using flotation method for detection of protozoan. It was observed that Eimeria, Entamoeba cysts and Giardia cysts were present with percentage prevalence of 93, 71 and 3% respectively by using the flotation technique. Species of Eimeria were also analyzed by flotation technique and it was resulted that Eimeria arloingi (4%), Eimeria intricate (8%) and Eimeria ahasta had 19% whereas Eimeria parva and ovinidalis were also observed in samples. Another similar research was conducted in Saudi Arabia on species of Eimeria by using flotation technique (Ibrahim 2012). This study revealed that Eimeria ovinidalis was observed in only 5% of faecal samples collected from goats. Eimeria ahasta had highest prevalence (56%) among other species.

Results of analysis indicated that Eimeria (31%) and Entamoeba histolytica (13%) were identified with high prevalence than other protozoans of gastrointestinal tract. Eggs of *Balantidium coli* and *Neospora* spp. were detected in faecal samples with percentage prevalence of 7 and 4% respectively (Fig. 3). Oocysts of Cryptosporidium and Giardia were not observed. Species of *Eimeria* were identified by using centrifugation technique. Eimeria ahasta oocysts were identified with its high prevalence of 20% in faecal samples of goat. By centrifugation method oocysts of *Eimeria intricate* was also observed in 8% samples but on the other hand *Eimeria arloingi* was detected in 3% faecal samples. Eggs of Eimeria parva and Eimeria ovinidalis were not observed by centrifugation method in faecal samples from goats. Summary of data obtained by different technique is tabulated in Table 1. In present research Eimeria was observed with prevalence percentage of 31% and Entamoeba *histolytica* (13%) followed by *Balantidium coli* (7%) and Neospora spp., (4%). However, Giardia and Cryptosporidium were not found in any samples. Research work was done with similar results by previous scientists (Zvinorova et al. 2016; Khodakaram-Tafti and Hashemna 2017; Sebba et al. 2021) who determined that Eimeria had high prevalence of (6.4 %) followed by Entamoeba histolytica (6%) and Balantidium coli 4.3% by using centrifugation method. Results of present study made it clear that female (32%) were more infected with faecal protozoan parasites compare to male (24%) animals as research work described the same analysis (Zvinorova et al. 2016; Khodakaram-Tafti and Hashemna 2017; Sebba et al. 2021) (Fig. 4).

It was concluded that goats of Lahore, Pakistan have gastrointestinal (GI) protozoan as they were observed

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in fresh feacal samples obtained from gastrointestinal tract (rectum). The percentage showed the rate of infection in goats. There is an adverse effect of parasitic load on cattle industry as well as public health. By this study we concluded the percentage of these protozoan in goat in Punjab, which will enhance the awareness in cattle owners for the control of parasitic infection.

Protozoan Parasites	Direct Smear Method			Flotation Technique				Centrifugation Method				
	Male=50		Female=50		Male=50		Female=50		Male=50		Female=50	
	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve
Eimeria spp	44	6	41	9	43	7	41	9	43	7	41	9
(31%)												
Entamoeba	47	3	47	3	47	3	46	4	47	3	46	4
histolytica												
(13%)												
Balantidium coli	47	3	48	2	48	2	48	2	48	2	47	3
(7%)												
Neospora spp	50	-	49	1	50	-	46	4	49	1	47	3
(5%)												
Giardia	50	-	50	-	48	2	48	2	50	-	50	-
Cryptosporidiu	50	-	50	-	47	3	47	3	50	-	50	-
m												
Total No. of												100
Samples												

 Direct Smear Method
 Flotation Technique
 Centrifugation Method

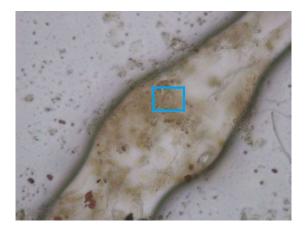


Fig. 1: Eggs of *Eimeria is* seen under 4X objectives of microscope by using flotation technique

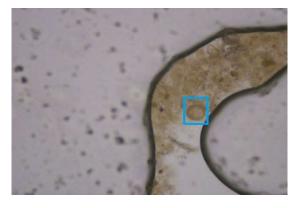


Fig. 2: Eggs of *Neospora* species as seen under 4X objectives of microscope by using flotation technique

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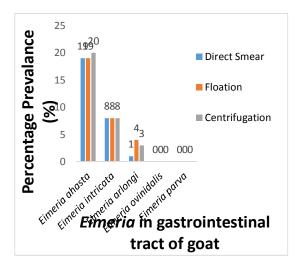


Fig. 3: Prevalence of *Eimeria* spp., in faecal samples collected from gastrointestinal tract of goat by different technique

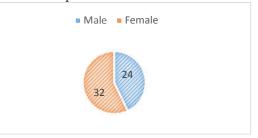


Fig. 4: Gender wise comparison for percentage prevalence of infected male and female

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