

## Prevalence, Hematology and Treatment of *Balantidium coli* among Small Ruminants in and Around Lahore, Pakistan

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### Abstract

Sheep and goats of Lahore district were examined for the prevalence, hematology and treatment of *Balantidium coli*. A total of 752 sheep and goats were examined (n=376 sheep; n=376 goats). Sheep had a somewhat greater prevalence (3.99%) than goats (3.46%). In both species, decrease in hemoglobin (Hb) and increased packed cell volume (PCV) was observed in infected animals. Efficacy of Secnidazole was higher as compared to Oxytetracycline and Metronidazole in all experimental animals. Balantidiasis is rarely studied infection in small ruminants and this is the first report published on the disease from Pakistan. It is still unclear whether this is an emerging infection, or whether it is a newly reported endemic in the studied area.

**Keywords:** *Balantidium coli*, Small ruminants, Treatment, Prevalence, Hematology, Oxytetracycline, Metronidazole

## Lahore, Pakistan ve Çevresinde Küçükbaş Geviş Getiren Hayvanlar Arasında *Balantidium coli*'nin Prevalansı, Hematolojisi ve Tedavisi

### Özet

Lahore ilçesindeki koyunlar ve keçiler *Balantidium coli*'nin prevalansı, hematolojisi ve tedavisi açısından incelendi. Toplam 752 koyun ve keçi incelendi (n=376 koyun; n=376 keçi). Keçi (% 3.46) ile karşılaştırıldığında koyunda daha fazla *B. coli* prevalansı (3.99%) gözlemlenmiştir. Her iki türde, enfekte hayvanlarda hemoglobin (Hb) değerinde azalma ve packed cell volumde (PCV) artma gözlemlenmiştir. Tüm deney hayvanlarında Oksitetrasiklin ve metronidazol ile karşılaştırıldığında seknidazol etkinliği daha yüksekti. Blantidiasis küçükbaş geviş getiren hayvanlarda nadiren çalışılmış bir enfeksiyondur ve Pakistan'dan bu ilk rapordur. Bu enfeksiyonun bu bölgede endemik olup olmadığı ya da artarak gözlenip gözlenmediği henüz bilinmemektedir.

**Anahtar sözcükler:** *Balantidium coli*, Küçükbaş geviş getiren hayvanlar, Tedavi, Prevalans, Oxytetracycline, Hematoloji, Metronidazole

### INTRODUCTION

Parasitism is not only a burden on small ruminant farmers nationwide but also a contributing factor in the reduction of per animal yield in Pakistan. It is one of the key threats for domestic animals, and is one of the primary obstacles to the development of a productive small ruminant agronomy [1]. Balantidiasis is a contagious malady worldwide [2]; it is most common in tropical and subtropical regions. In Tanzania, 4.8% *B. coli* prevalence was reported in goats [3], while in Kenya, its prevalence was reported to be between 2% and 3% in sheep and goats,

mostly as mixed infection with other parasites [4].

*B. coli* infection creates zoonotic hazards [5]. This large ciliated protozoon not only infects humans but nonhuman primates also [5]. Human balantidiasis is more common in poorly sanitized areas where human live in close contact with sheep, goats and pigs. It is chiefly spread through solid food and water that has been contaminated by human or animal feces containing *B. coli* cysts [6]. Hence it has also been considered as an emergent zoonotic pathogen [7].

There is a large body of knowledge on balantidiasis across pig raising nations but little research has been



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conducted on the parasite in small ruminants. This study elaborates the prevalence and chemotherapy of *B. coli* in small ruminants in Lahore district, Pakistan and will provide help in further studies on *B.coli* in small ruminants.

## MATERIAL and METHODS

About 5 g of fecal sample was collected directly from the rectum of 752 animals (n=376 sheep; n=376 goats). Self-sealing polythene bags were used to place samples in; these were transferred to the laboratory in ice pack cooler. The Sedimentation and Direct Smear Methods were used to find the *B. coli* presence while cystic count was carried out by the McMaster Technique. Briefly, direct smear technique was performed by a small quantity of feces was mixed with the physiological saline solution in a Petri dish and a uniform suspension was made by stirring. A drop of suspension was placed on glass slide, covered with cover slip and examination low power magnification (10x) of microscope for the presence of *B. coli* cyst. Sedimentation method was done by 5 grams of feces was thoroughly mixed with forty times of its volume of water. The suspension was then filtered through the fine sieve in a centrifuge tube and was then centrifuged at 1.500 rpm for 5-10 min. The supernatant was discarded. A small quantity of the retained sediment was then withdrawn into a pipette and transferred to a glass slide and examined under low power (10x) of microscope. In Mac Master Technique, 3 grams of feces was put in a shaker jar having 42 ml saturated NaCl. The jar was shaken well then poured through a wire mesh with an aperture of 0.15 mm. The filtrate of feces was well mixed and sufficient amount was withdrawn with a pasture pipette and then run into one counting chamber. After further mixing, a second sample was withdrawn and run into other counting chamber. All the cycts under the two separate grids were counted. Since 3 g of feces produced 45 ml of suspension (1 g per 15 ml suspension) and the volume of suspension examined was 0.3 ml (0.15 ml under each grid of the counting chamber) the number of eggs per gram of feces was obtained by multiplying the total number of eggs under the two grids by 50.

### Chemotherapeutic Trial

Of 24 small ruminants (n = 12 sheep; n = 12 goat) positive for *B. coli* were divided into three groups (A-C). Each group comprised of 8 (n = 4 sheep; n = 4 goat). Group A, B, and C were treated with a single oral dose of secnidazole at 10mg/kg, metronidazole at 25 mg/kg, oxytetracycline at 8mg/kg respectively. The fecal samples of all groups were collected at day 0 (pre-treatment) and then on day 2, 4, 6 and 12 (post-treatment). The efficacy of the drugs used was assessed on the basis of reduction in cystic count and was calculated as per formula of [8].

$$\left[ \frac{\text{Pretreatment CPG} - \text{Post treatment CPG}}{\text{Pretreatment CPG}} \right] * 100$$

### Hematological Studies

Hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), and total leukocyte count (TLC) were estimated by hematological analyzer. Blood samples (5ml) were taken from 10 animals (n=5 sheep; n=5 goat) positive for *B. coli* and 10 from healthy animals (n=5 sheep; n=5 goat) directly from the jugular vein in EDTA coated vaccutainers. The study was approved by the Ethics Committee of University of Veterinary and Animal Sciences Lahore Pakistan (Serial No. 171/2013)

### Statistical Analysis

Prevalence of *B. coli* was estimated by Pearson's chi-square test for significance whereas data on hematology was analyzed by Student's *t*-test. Data on chemotherapy was analyzed by one-way ANOVA, using SPSS (statistical package for social science).  $P < 0.05$  was considered as significant.

## RESULTS

Data on sex and breed vs. prevalence is shown in [Table 1](#). Of 752 animals surveyed, balantidiasis were found in 3.72%. Specie-wise prevalence revealed that *B. coli* were more prevalent in sheep (3.99%) as compared to goat (3.46%). However species risk factor was statistically found insignificant ( $P > 0.05$ ). The prevalence of *B. coli* in female sheep and goats were higher as compared to male. Amongst sheep population a high prevalence of *B. coli* was found in mix breeds (4.9%), followed by Thali (4.12%), Kajli (3.88%) and Salt Range (2.7%), respectively while in goats the prevalence was highest in mix breeds (4.38%), followed by Teddy (3.25%) and Beetal (2.59%), respectively. Data regarding hematology is presented in [Table 2](#). A significant ( $P < 0.05$ ) decrease in hemoglobin and increased PCV was observed in infected animals as compared to healthy ones. Secnidazole showed higher efficacy in sheep (86.66%) and goats (93.75%) as compared to metronidazole and oxytetracyclin at the end of treatment trial ([Table 3](#)).

## DISCUSSION

Prevalence of balantidiasis in sheep are in close agreement with the findings of Kanyari et al.<sup>[4]</sup> who recorded a 2% prevalence of *B. coli* in sheep and 3% in goats, reared in urban and peri urban areas of Africa. This slight variation in the prevalence might be due to the different geoclimatic and management conditions. However no significant difference ( $P > 0.05$ ) was observed among sexes in both species. Unlikely, Kanyari et al. noticed higher prevalence of balantidiasis in male small ruminants as compared to female ones <sup>[4]</sup>. However Khan et al.<sup>[9]</sup>. noted a statistically insignificant higher prevalence of *B. coli* in female donkeys as compared to males. In current study, mixed breeds showed higher prevalence of *B. coli*

**Table 1.** Sex and Breed-wise prevalence of *B. coli* in sheep and goats**Tablo 1.** Koyun ve keçilerde seks ve cinsine göre *B. coli*'nin prevalansı

Animals			Positive		Negative	95% CL	MH Chi-sq P Value	OR/Reciprocal
			n	%				
Sheep	Sex	Male	7	3.59	188	1.58- 6.97	-	OR = 0.81 [reciprocal = 1.24]
		Female	8	4.42	173	2.07- 8.22		
		Overall	15	3.99	361	2.34-6.35		
	Breed	Kajli	4	3.88	99	1.25-9.10	P = 0.934	-
		Salt range	2	2.70	72	0.46 to 8.64		
		Thali	4	4.12	93	1.32 to 9.65		
		Mix breed	5	4.90	97	1.82-10.53		
Goat	Sex	Male	7	3.27	207	1.44 to 6.36	-	0.88 [reciprocal = 1.14]
		Female	6	3.70	156	1.51 to 7.54		
		Overall	13	3.46	363	1.94 to 5.69		
	Breed	Teddy	4	3.25	119	1.04 to 7.66	P = 0.607	-
		Beetal	3	2.59	113	0.66 to 6.88		
		Mix breed	6	4.38	131	1.79 to 8.88		

**Table 2.** Effect of *Balantidium coli* on various blood parameters in sheep and goats (Mean  $\pm$  SD)**Tablo 2.** Koyun ve keçilerde çeşitli kan parametreleri üzerine *Balantidium coli*'nin etkisi (Mean  $\pm$  SD)

Hematological Parameters	Healthy Sheep	Affected Sheep	P-Value	Healthy Goats	Affected Goats	P-Value
Hemoglobin (g/dl)	11.64 $\pm$ 1.33	9.05 $\pm$ 0.92	0.009*	11.30 $\pm$ 1.01	9.49 $\pm$ 0.43	0.012*
PCV (%)	32.01 $\pm$ 1.14	34.05 $\pm$ 0.64	0.012*	32.03 $\pm$ 1.25	34.27 $\pm$ 0.62	0.012*
TEC (10 <sup>12</sup> /l)	12.26 $\pm$ 1.32	9.54 $\pm$ 0.96	0.007*	12.36 $\pm$ 1.04	9.736 $\pm$ 0.46	0.003*
TLC (10 <sup>9</sup> /l)	9.48 $\pm$ 1.24	10.50 $\pm$ 0.46	0.143	8.44 $\pm$ 1.02]	9.51 $\pm$ 0.53	0.084

\* Significant (P<0.05)

**Table 3.** Comparative efficacy of oxytetracycline, secnidazole and metronidazole at various days in sheep and goats (Mean  $\pm$  SD)**Tablo 3.** Koyun ve keçilerde çeşitli günlerde oksitetrasiklin, seknidazol ve metronidazol karşılaştırmalı olarak etkinliği (Ortalama  $\pm$  SS)

Group	Treatment	<i>B. coli</i> Cyst Count/g at Day in Sheep						<i>B. coli</i> Cyst Count/g at Day in Goats					
		0	2	4	6	12	P-value	0	2	4	6	12	P-value
A	Secnidazole	375 $\pm$ 95.74	275 $\pm$ 95.74	175 $\pm$ 95.74	100 $\pm$ 81.65	50 $\pm$ 57.74	0.152	400 $\pm$ 81.65	275 $\pm$ 95.74	200 $\pm$ 81.65	100 $\pm$ 81.65	25 $\pm$ 50	0.095
B	Metronidazole	400 $\pm$ 81.65	325 $\pm$ 50	300 $\pm$ 81.65	275 $\pm$ 95.74	225 $\pm$ 125.83		450 $\pm$ 57.74	350 $\pm$ 57.74	325 $\pm$ 95.74	250 $\pm$ 129.01	225 $\pm$ 95.74	
C	Oxytetracycline	375 $\pm$ 95.74	300 $\pm$ 81.65	250 $\pm$ 57.74	150 $\pm$ 57.74	125 $\pm$ 95.74		375 $\pm$ 50	300 $\pm$ 81.65	175 $\pm$ 95.74	150 $\pm$ 57.74	100 $\pm$ 81.65	

among sheep and goats. It was observed that the mix breeds are more susceptible to *B. coli* in both species, but breed was found to be an insignificant (P>0.05) risk factor in both species. Result of hematological values in this study suggests parasitic infestation induces blood loss via intestinal hemorrhaging. Similarly, these hematological signs have also been observed in *B. coli* infected pigs [10] and donkeys [9]. Blantidiasis causes intestinal pathological lesions, accompanied by severe hemorrhaging and bloody diarrhea. Analysis of CPG pre and post treatment in both species concluded that Secnidazole was the most effective at decreasing fecal cyst counts, followed by Oxytetracycline and Metronidazole, respectively. However, differences of the three drugs in lowering cyst counts was found statistically insignificant (P<0.05). There was lack of

literature mentioning the reasons for variations observed in treatment of balantidiasis in small ruminants. However, therapeutic results are closely in line with that of Bilal et al. [11] who also recorded significant decrease of *B. coli* cyst count in a positive cattle group receiving Secnidazole followed by Oxytetracycline and Metronidazole, respectively. Lowering in cyst count using Secnidazole revealed its higher efficacy as compared to Oxytetracycline and Metronidazole in sheep and goats. Previous studies also have demonstrated that Secnidazole and Oxytetracycline are more efficacious against *B. coli* in donkeys [9], cattle [4], and Buffalos than Metronidazole [12,13].

In recent years, the zoonotic importance of *B. coli* has been well noted and is especially detrimental to those

humans suffering from HIV/AIDS <sup>[14]</sup>. In Pakistan and other countries like Pakistan, small ruminants are mostly reared along with other animals and most of times are in close contact with human population. So, small ruminants infections in unhygienic environments may easily result on *B. coli* outbreaks in other animals and humans. So, clean drinking water and separate housing for domestic ruminants is advised to avoid transmission of infection and for securing public health. This is the first report from the studied area so it is difficult to conclude that either this is an emerging infection, or an endemic one that has been recently uncovered.

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