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Prevalence and Therapeutic Efficacy of Balantidiasis in Cattle in Nangarhar Province, Afghanistan

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ABSTRACT

Balantidiasis is a widespread protozoan parasitic disease that significantly impairs livestock productivity, particularly in cattle. This study investigated the prevalence of balantidiasis and evaluated the efficacy of therapeutic agents in cattle across ten districts of Nangarhar province, including Khogyani, Pachiragam, Chaparhar, Surkhrod, Hisarak, Behsud, Kama, Khiwa, Goshta, and Mohmand Dara, between March 2022 and March 2023. A total of 384 fecal samples were collected and analyzed using sedimentation and flotation techniques, revealing that 130 samples (33.8%) were positive for balantidiasis. District-wise prevalence rates were as follows: Khogyani 31.5%, Pachiragam 26.0%, Chaparhar 23.6%, Surkhrod 36.8%, Hisarak 23.0%, Behsud 41.0%, Kama 38.4%, Khiwa 43.0%, Goshta 46.0%, and Mohmand Dara 30.7%. Age-wise analysis indicated the highest prevalence in calves aged 1 to 6 months ($P < 0.01$), while female cattle were significantly more affected than males ($P < 0.01$). The study further assessed the efficacy of three therapeutic agents. Oxytetracycline (8 mg/kg) reduced oocysts per gram (OPG) by 26.1%, 36.9%, 38.0%, and 50.7% on days 3, 7, 14, and 21 respectively. Metronidazole (25 mg/kg) achieved reductions of 40.6%, 45.7%, 49.1%, and 47.4%, whereas the herbal preparation Shatra (35 mg/kg) produced reductions of 12.3%, 24.6%, 43%, and 46.2% over the same intervals. Statistical analysis indicated that metronidazole demonstrated the highest therapeutic efficacy. In conclusion, balantidiasis remains a significant parasitic threat in Nangarhar, especially affecting young calves and female cattle. Among the treatments tested, metronidazole shows the greatest potential for controlling the disease and improving livestock productivity.

Keywords: Balantidiasis; Cattle; Metronidazole; Oxytetracycline; Shatra

INTRODUCTION

Livestock play a vital role in the economic structure of a country and are essential for meeting human nutritional needs (Ahmed et al., 2020; Tarrar et al., 2008). Among livestock, cattle make a particularly significant contribution to national economic development. However, parasitic diseases remain a major obstacle to the growth of the livestock industry, especially in developing countries such as Afghanistan. These infections, affecting both ruminant and non-ruminant animals, may be caused by internal or external parasites. One of the most common parasites affecting both groups is *Balantidium coli* (Ahmed et al., 2020). *Balantidium coli*, the causative agent of balantidiasis, is typically found in the large intestine of healthy animals, where it usually exists in a non-pathogenic state. However, under conditions of stress or immunosuppression, it can become pathogenic and cause clinical disease (Chandra Roy et al., 2011), the increasing spread of such parasitic infections is largely attributed to limited awareness of parasite control measures and unfavorable environmental conditions.

Among parasitic infections, protozoan diseases hold significant importance in all ruminant species and can also affect non-ruminants. Balantidiasis is one of the most important protozoan infections affecting sheep, goats, cattle, and buffaloes, and it can also infect pigs, primates, humans, and other animals (Ahmed et al., 2020; Chandra Roy et al., 2011). *Balantidium coli* is a ciliate protozoan that typically resides in the large intestine under normal conditions but may become pathogenic under certain circumstances (Chandra Roy et al., 2011). The genus *Balantidium* belongs to the subphylum Ciliophora and the order Trichostomatida(Ponce-Gordo & Jirků-Pomajbíková, 2017). Although several species within this genus have been described, their taxonomic validity remains controversial (Verster, 1994). Some species have been differentiated based on host specificity, while others are classified by morphological

characteristics such as shape and size (Quadri & Navarathnam, 1966).

Among the known species, *Balantidium coli* and *Balantidium suis* were first isolated from pigs by McDonald in 2006. Both exhibit elongated, straight bodies, and the distinction between the two species has been challenged by (Levine, 1961). who reported no significant morphological differences between *B. coli* and *B. suis*. *Balantidium coli* is commonly found in various mammals (Quadri & Navarathnam, 1966), and morphological differences between the species were specifically studied in camels. This parasite has been reported to cause significant damage in regions such as northern Russia, Norway, Central and South America, Pacific Islands, and Finland. However, it is mainly restricted to equatorial and sub-equatorial zones, with severe outbreaks recorded in New Guinea (Van Der Hoeven & Rijpstra, 1957).

The life cycle of *Balantidium coli* comprises two developmental stages: trophozoite and cyst. Trophozoites primarily colonize the cecum and rectum (Urquhart, 1996). The trophozoite is the active, motile stage, characterized by two nuclei and a body covered with cilia. These trophozoites are excreted into the environment through the feces of infected animals. Infection is established when a susceptible host ingests contaminated food or water containing these stages, allowing the parasite to migrate to the large intestine (Chandra Roy et al., 2011). Reproduction occurs via binary fission (asexual) or conjugation (sexual). Trophozoites attach to the intestinal lining and cause colitis by producing the enzyme hyaluronidase, which facilitates mucosal invasion and triggers inflammation (Tempelis & Lysenko, 1957; Yazar et al., 2004). Clinically, this results in diarrhea and dehydration, accompanied by weight loss, weakness, and reduced productivity, leading to economic losses that affect both individual farmers and the broader national economy. Balantidiasis is a zoonotic disease and can be transmitted to humans, typically from pigs, via the fecal-oral route (Schuster & Ramirez-Avila, 2008). Microscopically, trophozoites or cysts of *Balantidium coli* can be detected in fecal samples using a 10× objective lens or by biopsy through a sigmoidoscope (Sampurna, 2007).

Afghanistan, with its fragile economy and heavy reliance on agriculture and livestock, depends largely on animal farming for livelihood and income. Since a large part of the population is involved in livestock production, balantidiasis poses a serious threat by reducing animal productivity and causing economic losses. Although this disease likely exists in other provinces, its high prevalence in dairy cattle in Nangarhar province suggests that livestock in this region may be particularly vulnerable. Therefore, this study was conducted to investigate the prevalence of balantidiasis and evaluate the effectiveness of treatment strategies in cattle in Nangarhar province, Afghanistan.

MATERIALS AND METHODS

Sample Size and Sampling Method

This study was conducted on cattle from ten randomly selected districts of Nangarhar Province. The sample size was calculated at a 95% confidence level with a 5% margin of error, assuming a disease prevalence of 50%, using the following formula:

$$\textbf{Sample Size} = \frac{Z_{1-\alpha/2}P(1-P)}{d^2}$$

As a result, a total of 384 cattle were included in the study. To obtain samples from a geographically dispersed population, a two-stage proportionate cluster random sampling technique was employed.

Independent Variables

This study examined the association between the prevalence of *Balantidium coli* infection and potential risk factors. Data on these factors were collected using a well-structured, pre-tested questionnaire. The variables considered in the analysis included the sex and age of the animals.

Dependent variables

The prevalence of *Balantidium coli* infection (i.e., whether cattle is infected or not, or the level of infection measured by oocysts per gram).

Collection of Fecal Samples

Approximately 5 grams of fecal sample were collected directly from the rectum of each animal using disinfected gloves. Each sample was placed in a self-sealing polythene bag, stored in ice containers, and transported to the Parasitology Laboratory, Faculty of Veterinary Sciences, Nangarhar University, for analysis. All samples were refrigerated for less than 48 hours prior to testing.

Fecal Sample Analysis

The fecal samples were examined using: Direct smear method for preliminary screening and McMaster technique to quantify infection intensity as Oocysts Per Gram (OPG), following Soulsby (1982), microscopic identification of *Balantidium coli* oocysts was performed as described by (Soulsby, 1968).

$$P = \frac{\text{No.of animals with the disease}}{\text{Total no.of Animals}} * 100$$

P= Prevalence

McMaster Technique Procedure

A 5 g fecal sample was mixed with 30 mL of saturated sodium chloride solution. The mixture was then filtered through a 15 mm sieve into a separate container and allowed to settle for 30 minutes. Oocysts, being lighter in specific gravity, floated to the surface. A 2–3 mL aliquot from the top layer was collected using a pipette and carefully placed into both chambers of a McMaster slide. Oocysts were counted in triplicate for each sample. The average number of oocysts counted was multiplied by 100 to determine the oocysts per gram (OPG) using the formula:

OPG = N × 100

Where **N** is the average number of oocysts observed.

Drug and Herbal Treatment Efficacy Evaluation

Twenty naturally infected cattle were randomly assigned to four groups, each consisting of five animals. Treatments were administered for three consecutive days. Fecal samples were collected on Day 0 (pre-treatment) and subsequently on Days 3, 7, 14, and 21 post-treatments. Oocyst counts per gram (OPG) were determined using the McMaster technique as described by Soulsby (1982). The efficacy of each drug was evaluated by calculating the percentage reduction in OPG, following the formula outlined by (Varady, Konigova, & Corba, 2004).

Group	Treatment	Dose	Route
A	Oxytetracycline (Rimoxyn, PDH Lab)	8 mg/kg	IM
B	Metronidazole (Flagyl, RPR Pvt Ltd)	25 mg/kg	Oral
C	*Shahtra* (*Fumaria parviflora*)	35 mg/kg	Oral
D	Untreated	—	Con

The treatment groups were as follows:

Statistical Analysis

Descriptive statistics were used to summarize the prevalence of *Balantidium coli* and associated variables. Independent samples t-tests were employed to assess significant differences in prevalence between categorical groups. One-way analysis of variance (ANOVA) was conducted to evaluate differences in drug efficacy among the treatment groups. All statistical analyses were performed using SPSS version 20.0, with a significance level set at $P < 0.05$.

Efficacy of drug (ED)= pretreatment OPG – Post treatment OPG x 100

ED= pretreatment OPG – Post treatment OPG*100

RESULTS

This study on the prevalence of balantidiasis in cattle and the evaluation of effective treatments was conducted from March 2022 to March 2023 across ten districts of Nangarhar Province: Khogyani, Pachiragam, Chaparhar, Surkhrod, Hisarak, Behsud, Kama, Khiwa, Goshta, and Mohmand Dara. Samples were collected from cattle owned by local livestock keepers, which were maintained at home and allowed to graze on pastures. None of these animals had undergone any specific deworming program prior to the study.

Total Incidence of Balantidiasis in Nangarhar Province

A total of 384 fecal samples were collected from cattle of various ages and sexes across the ten districts. Of these, 130 samples (33.8%) tested positive for balantidiasis **(Table 1).** Among the districts, Goshta exhibited the highest prevalence at 46%, whereas Hisarak recorded the lowest at 23%. The mean oocyst count per gram (OPG) across positive samples was 580**.**

Table 1. Incidence of *Balantidium coli* Infection in Cattle at Nangarhar Province

Animal	OPG (Mean ± SE)	No. Examined	No. Positive	Prevalence (%)
Cattle	580 ± 32.3	384	130	33.8

Prevalence of Balantidiasis in Cattle from Different Districts

Incidence of Balantidiasis in Cattle from Khogyani District

In this district, two groups of cattle differing in age and sex were studied. A total of 38 cattle were included, comprising 14 calves aged between one and six months and 24 animals older than six months. The sample was evenly divided by sex, with 19 males and 19 females. Among the 38 samples tested, 12 (31.5%) were positive for balantidiasis. Age-related analysis showed that 5 out of 14 calves (35.7%) tested positive, compared to 7 out of 24 older cattle (29%), indicating a higher infection rate in younger animals. Regarding sex, a significantly higher prevalence was observed in female cattle ($P < 0.01$) **(Table 2)**, with 4 of 19 males (21%) and 9 of 19 females (47%) testing positive.

Table 2. Prevalence by Age and Sex – Khogyani District

Category	OPG (Mean ± SE)	Prevalence (%)	Positive Samples	Total Samples
Age: 1–6 months	450 ± 21.34^{b}	35.7	5	14
Age: >6 months	490 ± 28.4^{a}	29.0	7	24
Sex: Male	390 ± 18.2^{b}	31.0	4	19
Sex: Female	550 ± 31.8^{a}	57.0	9	19

Data is presented as percentages and Mean ± SE. Significantly higher incidence was seen in 1–6 month-old calves and female cattle ($P < 0.01$).

Incidence of Balantidiasis in Cattle from Pachiragam District

In this district, two groups of cattle differing in age and sex were studied. Of the 38 cattle, 9 were calves aged between one and six months, while 29 were older than six months. The group included 19 males and 19 females. Among the 38 samples tested, 10 (26%) were positive for balantidiasis. Age-based analysis indicated that 3 of 9 calves (33.3%) tested positive, compared to 7 of 29 older cattle (24.1%), suggesting a higher incidence in younger animals. Sex-based analysis revealed a significantly higher infection rate in females ($P < 0.05$) **(Table 3),** with 4 of 19 males (21%) and 6 of 19 females (31.5%) testing positive.

Table 3. Prevalence by Age and Sex – Pachiragam District

Category	OPG (Mean ± SE)	Prevalence (%)	Positive Samples	Total Samples
Age: 1–6 months	403 ± 22.5^{b}	33.3	3	9
Age: >6 months	490 ± 25.8^{b}	24.13	7	29
Sex: Male	540 ± 31.5^{b}	21.0	4	19
Sex: Female	580 ± 33.4^{a}	31.5	6	19

The data is presented in percentages and as Mean ± SE (Standard Error). Based on sex, the incidence was significantly higher in female animals.

Incidence of Balantidiasis in Cattle from Chaprahar District

In Chaparhar district, two groups of cattle differing in age and sex were studied. Of the 38 collected samples, 9 were from calves aged between one and six months, and 29 were from cattle older than six months. The sample included 19 males and 19 females. Among these, 9 samples (23.6%) tested positive for balantidiasis. Age-wise analysis indicated that 3 of 9 calves (33.3%) tested positive, while 6 of 29 older cattle (20.7%) were positive. Regarding sex, 4 of 19 males (21%) and 5 of 19 females (26.3%) tested positive. Statistical analysis revealed a significant difference between the age groups, with a higher prevalence in older cattle ($P < 0.05$), whereas no significant difference was observed between males and females ($P > 0.05$) **(Table 4).**

Table 4. Prevalence by Age and Sex – Chaparhar District

Category	OPG (Mean ± SE)	Prevalence (%)	Positive Samples	Total Samples
Age: 1–6 months	490 ± 36.2[b]	33.3	3	9
Age: >6 months	560 ± 38.0[a]	20.69	6	29
Sex: Male	360 ± 32.1[a]	21.0	4	19
Sex: Female	390 ± 33.0[a]	26.3	5	19

The data is presented in percentages and as Mean ± SE (Standard Error). Based on age, the incidence was significantly higher among animals older than 6 months, while there was no significant difference based on sex.

Incidence of Balantidiasis in Cattle from Surkhrod District

In Surkhrod District, cattle were categorized into two age groups and by sex for the study. A total of 38 samples were collected, with 18 from calves aged 1–6 months and 20 from cattle older than 6 months. The sample included 19 males and 19 females. Among these, 14 samples (36.8%) tested positive for Balantidiasis. Age-wise analysis showed that 8 of 18 calves (44.0%) were positive, while 6 of 20 older cattle (30.0%) tested positive. Regarding sex, 5 of 19 males (26.3%) and 9 of 19 females (47.3%) were positive. Statistical analysis indicated a higher prevalence in calves compared to older cattle, and females exhibited a higher prevalence than males **(Table 5).**

Table 5. Prevalence and OPG of Balantidiasis in Cattle of Surkhrod District by Age and Sex

Category	OPG (Mean ± SE)	Prevalence (%)	Positive Samples	Total Samples
Age 1–6 months	200 ± 21.6	44.0	8	18
Age > 6 months	180 ± 20.1	30.0	6	20
Male	250 ± 32.1	26.3	5	19
Female	300 ± 33.5	47.3	9	19

Data presented as Mean ± Standard Error (SE). No statistically significant differences were found between age groups or sexes.

Incidence of Balantidiasis in Cattle from Hisarak District

A total of 38 cattle of varying ages and sexes from Hisarak district were examined to determine the prevalence of balantidiasis. Among these, 9 samples (23%) tested positive. Age-wise analysis showed that 3 of 10 cattle (30%) aged 1 to 6 months were positive, compared to 6 of 28 cattle (21%) older than 6 months. Regarding sex, 4 of 19 males (21%) and 5 of 19 females (26%) tested positive. Statistical analysis indicated a significantly higher prevalence in cattle older than 6 months ($P < 0.05$), whereas no significant difference was observed between males and females ($P > 0.05$) **(Table 6).**

Table 6. Prevalence of Balantidiasis in Cattle of Different Ages and Sexes in Hisarak District

Category (Group)	OPG (Mean ± SE)	Percentage (%)	Positive Samples	Total Samples
Age: 1–6 months	110 ± 14.9[b]	30	3	10

Category (Group)	OPG (Mean ± SE)	Percentage (%)	Positive Samples	Total Samples
Age: >6 months	330 ± 18.5[a]	21	6	28
Sex: Male	200 ± 24.6[b]	21	4	19
Sex: Female	250 ± 28.5[b]	26	5	19

Data are presented as percentages and Mean ± SE. Prevalence was significantly higher in animals older than 6 months, while no significant difference was found between males and females

Incidence of Balantidiasis in Cattle from Behsud District

In Behsud District, cattle were categorized by age and sex to assess the prevalence of Balantidiasis. A total of 39 samples were collected, including 15 from calves aged 1–6 months and 24 from cattle older than 6 months. The sample comprised 19 males and 20 females. Overall, 16 out of 39 samples (41%) tested positive for Balantidiasis. Age-wise analysis revealed that 6 of 15 calves (40%) and 10 of 24 older cattle (41%) were positive, with no significant difference between age groups ($P > 0.05$). Sex-wise, 7 of 19 males (36.8%) and 9 of 20 females (45%) were positive, with no statistically significant difference between sexes ($P > 0.05$) **(Table 7).**

Table 7. Prevalence of Balantidiasis in Cattle by Age and Sex in Behsud District

Category (Group)	OPG (Mean ± SE)	Prevalence (%)	Positive Samples	Total Samples
Age: 1–6 months	340 ± 22.9[b]	40.0	6	15
Age: >6 months	500 ± 29.7[a]	41.7	10	24
Sex: Male	380 ± 23.4[b]	36.8	7	19
Sex: Female	400 ± 28.5[a]	45.0	9	20

Data are presented as percentages and Mean ± SE. There was no significant difference between age groups or sexes in terms of balantidiasis prevalence.

Incidence of Balantidiasis in Cattle from Kama District

In Kama District, a total of 39 cattle of different ages and sexes were examined for Balantidiasis, with 15 animals (38.4%) testing positive. Among 18 samples from calves aged 1–6 months, 7 (38.8%) were positive, while 8 of 21 samples (38%) from cattle older than 6 months tested positive, showing no significant difference between age groups ($P > 0.05$). Analysis by sex revealed that 4 of 19 males (21%) and 11 of 20 females (55%) were positive, indicating a significantly higher prevalence in females compared to males ($P < 0.05$) **(Table 8).**

Table 8. Balantidiasis Cases by Age and Sex in Cattle of Kama District

Category	OPG (Mean ± SE)	Percentage (%)	Positive Cases	Total Samples
Age: 1–6 months	280 ± 18.9[b]	38.8	7	18
Age: >6 months	320 ± 20.5[a]	38.0	8	21
Sex: Male	140 ± 12.4[b]	21.0	4	19
Sex: Female	560 ± 31.2[a]	55.0	11	20

Data are presented as mean ± standard error (SE) and percentages. No significant difference was found based on age, but a significant difference was observed between female and male cattle.

Incidence of Balantidiasis in Cattle from khiwa District

In Khiwa district, fecal samples were collected from 39 cattle of varying ages and sexes, with 15 cases (43%) testing positive for balantidiasis**.** Among 10 samples from cattle aged 1 to 6 months, 5 (50%) were positive, while 10 out of 29 samples (34.4%) from cattle older than 6 months tested positive. Regarding sex, 7 out of 19 male cattle (36.8%) and 8 out of 20 female cattle (40%) were positive. A significantly higher prevalence was observed in cattle older than 6 months compared to younger animals ($P < 0.05$), while no significant difference was found between males and females ($P > 0.05$) **(Table 9).**

Table 9. Balantidiasis Cases by Age and Sex in Cattle of Khiwa District

Category	OPG (Mean ± SE)	Percentage (%)	Positive Cases	Total Samples
Age: 1–6 months	300 ± 17.3[b]	50.0	5	10
Age: >6 months	490 ± 22.4[a]	34.4	10	29
Sex: Male	200 ± 11.22[b]	36.8	7	19
Sex: Female	280 ± 14.22[a]	40.0	8	20

The data in the above table are presented as percentages and Mean ± SE. A significantly higher prevalence of balantidiasis was observed in cattle aged over six months.

Incidence of Balantidiasis in Cattle from Goshta District

In Goshta district, 39 cattle of varying ages and sexes were examined, with 18 (46%) testing positive for balantidiasis**.** Among 22 samples from cattle aged 1 to 6 months, 10 (45%) were positive, while 8 out of 17 samples (47%) from animals older than 6 months tested positive. Regarding sex, 7 out of 19 male cattle (36.8%) and 11 out of 20 female cattle (55%) were infected. The prevalence was significantly higher in cattle older than 6 months and in females compared to males ($P < 0.05$) **(Table 10).**

Table 10. Balantidiasis Cases by Age and Sex in Cattle of Goshta District

Category	OPG (Mean ± SE)	Percentage (%)	Positive Cases	Total Samples
Age: 1–6 months	490 ± 23.22[a]	45.0	10	22
Age: >6 months	320 ± 18.8[b]	47.0	8	17
Sex: Male	300 ± 14.1[b]	36.8	7	19
Sex: Female	500 ± 24.0[a]	55.0	11	20

The data in the above table are presented as percentages and Mean ± SE. Significant differences were observed in prevalence by age and sex, with higher rates in older and female cattle.

Incidence of Balantidiasis in Cattle from Mohmand dara District

In Mohmandara district, fecal samples from 39 cattle of different ages and sexes were examined, with 12 (30.7%) testing positive for balantidiasis**.** Among 14 samples from cattle aged 1 to 6 months, 5 (35.7%) were positive, while 7 out of 15 samples (46.6%) from cattle older than 6 months tested positive. Regarding sex, 4 out of 19 males (21.05%) and 8 out of 20 females (40%) were infected. The prevalence was significantly higher in cattle older than 6 months and in females compared to males ($P < 0.05$) **(Table 11).**

Table 11. Balantidiasis Cases by Age and Sex in Cattle of Mohmandara District

Category	OPG (Mean ± SE)	Percentage (%)	Positive Cases	Total Samples
Age: 1–6 months	200 ± 11.22[b]	35.7	5	14
Age: >6 months	310 ± 14.33[a]	46.6	7	15
Sex: Male	230 ± 12.34[b]	21.05	4	19
Sex: Female	400 ± 17.4[a]	40.0	8	20

The data in the above table are presented as percentages and Mean ± SE. Significant differences in prevalence were found based on age and sex, with older and female cattle showing higher infection rates.

Drug Efficacy

The efficacy of the treatments was assessed by measuring the reduction in oocyst counts per gram (OPG) in fecal samples after administration. The percentage reduction in OPG was calculated for each treated group and compared with the untreated control group. Group A received oxytetracycline intramuscularly at 8 mg/kg body weight, Group B was administered metronidazole orally at 25 mg/kg, and Group C received Shahtra (*Fumaria parviflora*) orally at 35 mg/kg. Fecal samples were collected and analyzed for OPG on days 3, 7, 14, and 21 post-treatments. Group D served as the untreated control, with OPG values monitored for comparison. At baseline (day 0), there were no statistically significant differences in mean OPG values among groups A, B, and C ($P > 0.05$). By day 3, groups A and B showed a significant reduction in OPG compared to the control ($P < 0.05$), and this significant decrease persisted through day 7 ($P < 0.05$). However, no significant differences in OPG

were observed among any of the four groups on days 14 and 21 ($P > 0.05$) **(Table 12).**

Table 12. Effect of different drugs on balantidiasis based on OPG (Mean ± SE)

Groups	OPG at Days (Mean ± SE)				
	0 Day	**3rd Day**	**7th Day**	**14th Day**	**21st Day**
A (Oxytetracycline)	650 ± 31.3^{a}	480 ± 27.6^{a}	410 ± 24.3^{b}	380 ± 23.8^{b}	320 ± 17^{b}
B (Metronidazole)	590 ± 31.2^{a}	350 ± 29.4^{b}	320 ± 28.2^{b}	300 ± 21^{b}	310 ± 15.5^{b}
C (Shahtra)	650 ± 33.5^{a}	570 ± 41.6^{b}	490 ± 39.5^{b}	370 ± 29.3^{b}	350 ± 37.2^{b}
D (Control)	450 ± 27.4^{d}	650 ± 34.3^{c}	690 ± 30.9^{a}	720 ± 35.4^{b}	850 ± 39.9^{a}

Values are expressed as Mean ± SE. Superscript letters (a–d) denote statistically significant differences among groups at each time point ($P < 0.05$).

Efficacy of Individual Treatments

Oxytetracycline (Group A), administered intramuscularly at 8 mg/kg, produced OPG reductions of 26.1%, 36.9%, 38%, and 50.7% on days 3, 7, 14, and 21, respectively, while the control group showed a progressive increase in OPG counts **(Table 13)**. Metronidazole (Group B), given orally at 25 mg/kg, demonstrated consistent efficacy with OPG reductions of 40.6%, 45.7%, 49.1%, and 47.4% over the same time points. Shahtra (Group C), a herbal remedy administered orally at 35 mg/kg, showed lower initial reductions of 12.3% and 24.6% on days 3 and 7, but its effectiveness increased to 43% and 46.15% by days 14 and 21, respectively, approaching the efficacy of the other treatments **(Table 13).**

Table 13. Percentage reduction in OPG following treatment with different drugs

Groups	OPG Pre-treatment	3rd Day (%)	7th Day (%)	14th Day (%)	21st Day (%)
A (Oxytetracycline)	650	26.1	36.9	38.0	50.7
B (Metronidazole)	590	40.6	45.7	49.1	47.4
C (Shahtra)	650	12.3	24.6	43.0	46.15

DISCUSSION

Incidence and Related Factors

Based on the results of this study, the prevalence of Balantidiasis varied across different districts of Nangarhar Province, with the highest prevalence recorded in Goshta District (46%) and the lowest in Hesarak District (23%). This notable variation in prevalence among districts may be influenced by climatic factors, particularly humidity, as higher prevalence was observed in warmer, more humid regions, while lower prevalence occurred in drier, colder areas. These findings align with a similar study by Roy et al. in Bangladesh, which reported a prevalence of *Balantidium coli* of 45.03% (Roy et al., 2011). Additionally, our findings align with a study in Mumbai, India, where the prevalence in regions with lower humidity was found to be 27.5% (Raote, Narsapur, & Niphadkar, 1994). Our results also closely match the study by Bilal et al., which reported a 25% incidence of *Balantidium coli* in cattle along the Ravi River in Lahore, Pakistan (Bilal et al., 2009). However, the results of this study differ from those of Patil et al., who found an incidence of 17.07% in cattle in India. This discrepancy may be attributed to factors such as breed, age, climatic variation, and the different living conditions of the animals (Patil et al., 1998).

Regarding animal age, our study observed a higher prevalence of *Balantidium coli* in older cattle compared to younger ones. This finding is consistent with the study by Mirzaei and Khovnd in Iran, which reported that 47.6% of cattle over 3 years old were infected with *Balantidium coli*, compared to 25.6% of cattle aged between 1 and 3 years (Mirzaei & Khovand, 2015). Similarly, Biswas et al. observed a higher incidence in adult cattle (44.44%) compared to younger cattle (42.65%) (Biswas, Alam, Riad, Hanif, & Ahmed, 2020). Our findings also correlate with those of Al Numan et al., who found a higher incidence in adult cattle (45.7%) compared to younger cattle (40.0%) in Bangladesh (Al Numan et al., 2022). On the other hand, a study in Iraq found no significant correlation between age and the incidence of Balantidiasis, although the highest incidence (13.15%) was observed in younger cattle (under 10 years old) compared to older cattle. This difference could be due to the age groupings used in the study, with the definition of younger cattle differing between studies.

Another important factor affecting the incidence of Balantidiasis is the animal's sex. The results of this study indicated a higher incidence of *Balantidium coli* in cows compared to bulls. This finding aligns with Biswas et al., who reported a higher incidence in female cattle (43.31%) compared to male cattle (31.51%) (Biswas et al., 2020). Similarly, studies conducted in Bangladesh also reported a higher incidence in female cattle (58.8%) compared to male cattle (45.8%). In a study in Mymensingh, Bangladesh, female cattle also showed a higher incidence (46.6%) compared to male cattle (42.2%) (Al Numan et al., 2022).

Treatment

Balantidiasis is a zoonotic protozoal disease that is widely distributed worldwide, with the highest incidence reported in domesticated pigs. However, the disease has also been reported in other animals, including cattle. The disease is diagnosed by collecting samples from the animal's feces and identifying the oocysts and trophozoites of the *Balantidium coli* parasite. Various treatments have been studied for the disease, with tetracycline, metronidazole, and paromomycin found to be effective in animals (Arslan Ahmed et al., 2020). Our study supports these findings, as the administration of oxytetracycline and metronidazole to the studied cattle resulted in a significant reduction in the OPG (Oocyst Per Gram) count of *Balantidium coli*. Similarly, Patil et al. reported that tetracycline was effective in treating Balantidiasis in cows (Patil et al., 1998). Bilal et al. also reported that oxytetracycline had an efficacy of 62%, while metronidazole showed an efficacy of 37.5% (Bilal et al., 2009). Jamil and Ijaz, found that oxytetracycline, metronidazole, and cisnidazole were effective in treating *Balantidium coli* in pigs and goats. Additionally, cisnidazole was found to be more effective than oxytetracycline and metronidazole (Jamil et al., 2015).

Tarrar et al. also studied the effects of cisnidazole and other drugs in cattle around Lahore, reporting that cisnidazole was effective in treating Balantidiasis (Tarrar et al., 2008). Other studies have similarly reported the effectiveness of cisnidazole, with efficacy rates of 89.5% in treating *Balantidium coli* (Khan et al., 2013). In addition to synthetic treatments, research has also focused on traditional plant-based remedies for protozoal diseases. For example, the plant *Fumaria parviflora* has shown protozoal activity, with

phytochemical analysis revealing the presence of glucosides, sterols, alkaloids, and fatty acids, which contribute to its anti-inflammatory and anti-protozoal properties (Jamaldeen et al., 2022). Our study also found that *Fumaria parviflora* had a significant effect in reducing *Balantidium coli* oocysts in cattle, with a gradual reduction in OPG count on days 3, 7, 14, and 21, showing reductions of 12.3%, 24.6%, 43.0%, and 46.15%, respectively. Other plant-based treatments have also been explored, such as the effects of Mella Azadarch and Nigella sativa. Tarrar et al. observed reductions of 16%, 33%, and 50% in *Balantidium coli* counts on days 3, 7, and 10, respectively, after administering Mella Azadarch (Tarrar et al., 2008). Khan et al. found that Nigella sativa reduced *Balantidium coli* counts by 40% (Khan et al., 2013).

CONCLUSION

Balantidiasis is a prevalent protozoan parasitic disease in cattle across Nangarhar province, with the highest infection rates observed in young calves aged 1 to 6 months and in female cattle. The study demonstrated that all three tested therapeutic agents, oxytetracycline, metronidazole, and the herbal preparation Shatra, were effective in reducing oocyst counts, with metronidazole showing the highest therapeutic efficacy. These findings highlight the ongoing impact of balantidiasis on cattle productivity in the region and emphasize the importance of targeted treatment strategies, particularly the use of metronidazole, to improve livestock health and productivity. Future research should investigate the specific risk factors contributing to balantidiasis in cattle in Nangarhar, including management practices and environmental conditions. Studies could also evaluate the efficacy of combination treatment strategies to enhance parasite control and reduce infection rates.

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