

## COMPARATIVE EFFICACY OF THREE ANTHELMINTIC DRUGS AGAINST GASTROINTESTINAL PARASITES IN LACTATING COWS FROM LAHORE, PAKISTAN

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### ABSTRACT

The control of gastrointestinal (GIT) parasites remains critical for maintaining dairy herd health, productivity, and economic viability. This study evaluated the efficacy of three anthelmintic drugs Nilzan Plus, Ivermectin, and Albendazole on lactating cows. Forty cows were divided into four groups: A, B, and C (treatment groups), and D (control). Fecal samples were collected pre-treatment (day 0) and post-treatment (days 7 and 14). Fecal Egg Count (FEC) was monitored using the McMaster technique to assess treatment outcomes. Nilzan Plus showed the highest efficacy with a 70.85% reduction in FEC by day 7 and 98.57% by day 14. Ivermectin also demonstrated strong results, with a 69.45% reduction on day 7 and 94.28% on day 14. Albendazole was less effective, achieving only 43.07% FEC reduction on day 7 and 88.28% on day 14. Nilzan Plus eliminated *Toxocara spp.*, *Bovine Hookworm*, and *Moniezia spp.*, while Ivermectin effectively controlled *Haemonchus spp.* and *Bovine Hookworm*, though *Cooperia spp.* showed resistance. Albendazole was the least effective, failing to eliminate *Rumen Fluke*, *Cooperia spp.*, or *Ostertagia spp.* In conclusion, all drugs reduced parasitic infections to varying degrees, with Nilzan Plus proving most effective, followed by Ivermectin. Albendazole showed limited efficacy. It is concluded Incorporating proper hygiene and pasture management, along with strategic anthelmintic use, is essential for sustainable parasite control.

## 1. INTRODUCTION

Gastrointestinal parasites (GIPs) have continued to be one of the most significant issues in animal production in the world and especially in developing countries. The most common of these parasites are helminths like nematodes, cestodes, trematodes, which reduce the productivity and health of animals and impose substantial costs on livestock owners (Mia *et al.*, 2021; Pervin Heema, 2023). These can easily be identified in infected cattle as poor weight gain, stunted growth, reduced milk production, anemia, diarrhea and, in extreme cases, death (McFarland *et al.*, 2022).

Climate conditions, animal management procedures, pasture contamination, and the fitness of the host are some of the factors that typically determine the incidence of GIPs (Samad, 2019; Jittapalapong *et al.*, 2010). In South Asia and Sub-Saharan Africa, these problems are some of the most intense because of poor availability of veterinary diagnostic resources, insufficient knowledge base in the farmer population, and low levels of control strategies (Barua, 2016). In the past, parasitic diseases have been among the major causes of cattle losses, affecting large portions of the animal population. As a result, control strategies have relied almost exclusively on chemical anthelmintic drugs such as ivermectin, albendazole, and tetramisole (Islam *et al.*, 2015). Currently, these drugs have worked well over the decades, but the overuse of broad-spectrum medications has probably caused the

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development and proliferation of anti-drug strains of the parasites, therefore, rendering standard therapies ineffective (Kotze *et al.*, 2014). Exposure to the same drug will result in anthelmintic resistance since surviving genetically resistant individuals will have a chance to multiply. The issue has been sufficiently identified across several areas, and the answers to an alternative and sustainable control method cannot be unfolded quickly enough (Gianechini, 2024; Saeed and Alkheraije, 2023).

Among the most promising strategies is the Targeted Selective Treatment (TST) approach. TST is based on the observation that parasite loads in herds are skewed, with a few individuals carrying the majority of the parasites (Swarnakar *et al.*, 2015). TST does not involve mass deworming of all animals, but rather targets clinically afflicted, immunocompromised, or poorly performing individuals. This strategy reduces unnecessary drug use, slows the emergence of resistance, lowers treatment costs, and minimizes environmental contamination from chemical residues (Jackson, 2013). Additionally, TST promotes natural immunity by allowing healthy animals to be exposed to low parasite levels, thereby strengthening herd resilience over time. Mectizan and Albendazole, along with their degradants, pass through the digestive system and can contaminate water bodies and soil, adversely affecting non-target organisms such as dung beetles, beneficial insects, and aquatic species, ultimately destabilizing ecosystems (Huang *et al.*, 2014). In comparison, TST is less environmentally harmful and requires fewer chemicals. The success of TST and other targeted approaches depends on accurate diagnostics. Conventional methods such as Fecal Egg Counts (FEC) and Fecal Egg Count Reduction Tests (FECRT) remain valuable for assessing parasite burdens, although they are time-consuming, especially in large herds (Playford and Besier, 2024). Several commercial veterinary products continue to effectively control gastrointestinal parasites. For example, SELZAIN Drench, containing oxclozanide, levamisole, and cobalt sulfate, targets flukes and roundworms while providing essential micronutrients. Ivermectin derivatives, such as Ivermex, are widely used against internal and external parasites, though resistance must be monitored. Alzo-20, containing Albendazole, offers broad-spectrum activity and is suitable for integrated treatment

programs. Withdrawal periods should be strictly observed to ensure food safety and prevent harmful residues in milk and meat. Protozoan parasites, particularly *Cryptosporidium* and *Eimeria*, in addition to helminths, can cause serious disease in calves, including diarrhea, dehydration, and impaired growth. These are zoonotic pathogens that can infect humans via contaminated water, meat, or direct contact. Preventive measures include proper farm hygiene, biosecurity protocols, and provision of clean water (Otranto and Wall, 2024; McFarland *et al.*, 2022). The present study was done to assess the diversity and prevalence of gastrointestinal tract (GIT) parasites and to evaluate the efficacy of three anthelmintic drugs namely, Nilzan Plus, Ivermectin and Albendazole against GIT parasites in lactating cows.

## 2. MATERIALS AND METHODS

The present study was designed to analyze gastrointestinal parasites in fecal samples from 40 Lactating cows across different dairy farms. Samples were collected from four farms named Farm A, Farm B, Farm C and Farm D all located in the same area of Harbanspura Gawala Colony, Lahore. Laboratory analysis for parasite identification was carried out in the Parasitology Department of the University of Veterinary and Animal Sciences (UVAS) and at the Lahore Garrison University (LGU).

### Preparation and Examination of Fecal samples

The fecal matter was preserved in a 10% formalin solution consisting of 9 parts of formalin and one-part feces and stood at room temperature in the laboratory. The examination of fecal samples for the identification of gastrointestinal parasites was carried out using two approaches: physical examination and microscopic examination. During the physical examination, particular attention was given to the consistency of the fecal samples, and observations were recorded accordingly. Microscopic examination was performed using several diagnostic techniques, including the Direct Smear Method, Centrifugal Floatation Technique, Sedimentation Technique, and EPG/OPG estimation (McMaster Egg Counting Technique) for quantifying the eggs or oocysts present in the samples.

#### 1. Direct Smear Method

In the Direct Smear Method, a thin smear was prepared

by mixing a small amount of fresh fecal sample with two to three drops of 0.9 % saline solution on a clean, sterile, and grease- free glass slide. A toothpick was used to homogenize the mixture. As described by Foreyt (2013), a coverslip was placed gently over the smear to avoid the formation of air bubbles. The slide was examined under a light microscope (Olympus CX21) at 4X and 10X magnification to detect the presence of eggs/ova, larvae, and oocysts of gastrointestinal parasites. Each sample was analyzed in triplicates to ensure accuracy. This procedure aligns with recent recommendations for direct wet mount microscopy in parasitological diagnosis (Smith *et al.*, 2025).

## 2. Centrifugal Flootation Technique

In Centrifugal Flootation Technique, 3 grams of fecal sample was mixed thoroughly in 42 ml of water in a beaker. The solution was then poured through a nylon tea strainer to remove the debris. The filtrate was collected, mixed and transferred into a 15 ml Falcon tube. The tube was placed in the centrifuge and centrifuged at 1500 rpm for 10 minutes. After centrifugation, the supernatant was poured off, the sediment was agitated, and the Falcon tube was refilled to the previous level with saturated NaCl solution. The tube was again placed in the centrifuge for 10 minutes at 1500 rpm.

After this, the Falcon tube was placed on the rack, and the cap was opened. A few drops of NaCl solution were added to top up the tube to the brim. A coverslip was placed on top of the tube and left undisturbed for some time. The coverslip was then removed by lifting it vertically with a deliberate movement and placed on a clean glass slide. The slide was placed on the stage of a light microscope (Olympus CX21) and observed at 4×, 10×, and 40× magnification for the detection of parasitic eggs and oocysts of gastrointestinal parasites.

## 3. Sedimentation Technique

A 5-gram portion of each sample was diluted thoroughly in tap water. After several dilutions, the remaining sediment was retained. Using a dropper, a few drops of water from the upper surface of the sediment were transferred onto a clean glass slide. A coverslip was placed over the smear, and the slide was placed on the stage of the microscope. It was then examined under a light microscope at 10× magnification for the presence of eggs/oocysts of

various gastrointestinal parasites, as described by Soulsby (2015).

## 4. McMaster Egg Counting Technique

In the McMaster egg counting technique, eggs or oocysts of gastrointestinal parasites in ruminants were quantified following the method described by Soulsby 2015. A 100 ml glass beaker was filled with 35% sodium chloride solution upto the 28 ml mark. The prepared solution was then passed through a strainer to remove debris and collected in a clean beaker. After thorough mixing, the solution was drawn into a Pasteur pipette, and both counting chambers of the McMaster slide were filled with the prepared flotation solution (Cringoli *et al.*, 2004).

Eggs/oocysts per gram (EPG/OPG) were calculated using the following formula:

EPG = Number of Eggs/Oocysts counted in two chambers × 50

If the eggs/oocysts are present in only one chamber, then the following formula is used.

EPG = Number of Eggs/Oocysts counted in one chamber × 100

$$Prevalence \text{ (\%)} = \frac{\text{Number of animals positive at a particular point in time}}{\text{Number of animals examined at a particular point in time}} \times 100$$

## 3. RESULTS

This study investigated the prevalence of Gastrointestinal parasites in Lactating cows and efficacy of three selected anthelmintic drugs in treating these GIT parasites. The samples were collected from four different farms in Harbanspura, Lahore. For this cross-sectional study, 40 samples were collected, each from one lactating cow of varying age and mixed breed.

### Parasitological Detection and Egg Count Results by Multiple Diagnostic Methods

Several parasitological methods were used in this study to identify and measure the gastrointestinal parasites in the fecal samples. The Direct Smear Method was employed to rapidly screen fresh fecal samples, allowing immediate observation of motile stages of *Eimeria* (e.g., schizonts). Additionally, samples with high parasite burdens, such as

*Haemonchus* and *Cooperia*, could be promptly detected. While, Centrifugal Flotation Technique also improved the observation of light-dx eggs of Bovine Hookworm, *Toxocara*, *Moniezia*, *Haemonchus* and *Ostertagia* spp by agglomerating them at the surface, where they were more easily examined using a microscope. Techniques Conversely, the Sedimentation Technique was best in recovery of heavy eggs e.g. Rumen fluke (*Paramphistomum* spp.) and *Schistosoma* spp. that could have been lost during flotation. Lastly, McMaster Egg Counting Method allowed the study to quantitatively determine the intensity of infections based on estimating eggs per gram (EPG) of all identified species, and it ensures an objective form of comparison to determine the density of parasite so that it can be used to compare the burden of samples. By combining these complementary techniques, they were able to detect these parasites (both helminth and protozoan), as well as quantify them, on a wide variety at a sensitive level.

#### **Parasitic Prevalence in Pre-Treatment Samples**

The study was carried out on four cattle farms (A-D) with 40 fecal samples that were taken to determine the prevalence of gastrointestinal parasitic infections. The overall result indicated the variation in the distribution of parasites among farms. Farm D had been the most positive farm especially in Bovine hookworm (n=8), *Cooperia* spp. (n=3), Rumen fluke (n=7), and *Moniezia* spp. (n=5). Farm A exhibited high density of *Toxocara* spp. (n=6), moderate frequencies of Bovine hookworm detection (n=4), Rumen fluke (n=4) and *Moniezia* spp. (n=3). Of significance, *Trichostrongylus* spp., *Ostertagia ostertagi* and *Haemonchus contortus* were not found in Farm A. Farm B had a high load of Bovine hookworm (n=6), and occasional *Trichostrongylus* spp., *Ostertagia ostertagi*, *Haemonchus contortus* as well as *Moniezia* spp. Farm C was the lowest in parasite load with only a single encounter of Bovine hookworm, *Trichostrongylus* spp., and Rumen fluke 2 cases. *Schistosoma* spp. was only found in Farm A (n=1) and there were no mixed infections of more than three different parasites found in any of the farms. These results indicate the inconsistent parasitic loads among farms, where Bovine hookworm, *Toxocara* spp., Rumen fluke and *Moniezia* spp. are the widely identified parasites (Table 1).

#### **Parasitic Prevalence and Total Egg Count in Pre-Treatment Samples of different Farms**

The general pre-treatment parasitological statistics of four farms showed that gastrointestinal parasites among the presented animals were diverse and significant. Bovine Hookworm turned out to be the most prevalent and predominant parasite that was found at all farms with the most eggs number at Farm B (3000 EPG). Rumen Fluke was also highly distributed in all the farms, especially Farm D (1900 EPG) which showed an indication that it has a good environment to spread when conditions favor it. *Toxocara* and *Moniezia* were moderately widespread with the greatest values of EPG related to Farm A and lower values related to Farms B and C. *Haemonchus* was present in three out of four farms which is evidence of its functionality overall among livestock. Parasites like *Schistosoma* spp., and Schizonts of *Emiria* were less often found and were confined to Farm A only, an indication of a localized infection. Besides, *Cooperia* spp. and *Ostertagia* spp. were detected in Farms B, C and D, which was one more illustration of the variance in parasite types and intensity that farm environments reveal (Table 2).

#### **Parasitic Prevalence in Post-Treatment Samples**

Fecal egg count (FEC) reduction rates of Albendazole, Ivermectin, and Nilzan Plus after 7 and 14 days of treatment are given in the table. On Day 7, average reduction was highest in Nilzan Plus 70.85%, followed by Ivermectin 69.45% and Albendazole 43.07%. By the 14th day, all treatments achieved a significant improvement, whereby Nilzan Plus recorded the highest reduction (98.57%), Ivermectin (94.28%) and Albendazole (88.28%) respectively. The results showed that Nilzan Plus was the most effective treatment overall, next to Ivermectin, with Albendazole revealing a slower but continuing decline in parasite numbers (Table 3).

#### **Categorization of Samples Based on Fecal Consistency**

The fecal samples were further categorized based on consistency into three groups: normal (n=24), semi-solid (n=11), and diarrheic (n=5). Within these categories, the distribution and infection rates of trematodes specifically *Paramphistomum cervi* (Rumen Fluke) and *Schistosoma* spp., were assessed to evaluate their prevalence in relation to fecal

consistency. The most common trematode identified was Rumen Fluke, with the total infection being 47 percent. Diarrheic samples showed the highest rate of infection at 80% (4/5) followed by semi-solid samples at 36 percent (4/11) whereas the lowest was in the normal samples at 25 percent (6/24). These results mean that there is a positive relationship between the fecal looseness and the fluke burden implying that more serious cases of digestive upset in cattle may be precipitated by heavier infections. In one of the semi-solid samples, the presence of *Schistosoma* spp. was identified and showed the total infection rate of 3 % which states that *Schistosoma* spp. was relatively uncommon in the considered population (Table 4).

#### **Statistical Analysis of Parasitic Prevalence**

Data was analyzed for one-way ANOVA, by using SPSS version 23 and no significant statistical variations in parasite prevalence between different farms since the obtained p-value measured at 0.605. All farms showed comparable patterns when it came to parasite distribution. This suggests that parasite distribution is relatively similar among farms.

### **4. DISCUSSION**

The effectiveness of diverse anthelmintic interventions to manage dairy cow digestive parasite infections on a series of agricultural farms was studied. The research delivers essential details about treatment efficiency differences and how local specific elements affect parasite numbers together with their general influence on dairy herd functions and financial outlook. [Martinez et al., \(2023\)](#) reported the same, according to their findings, poor sanitation standards and hygiene conditions were the two major factors responsible for the transmission of gastrointestinal parasites in ruminants. The study showed decreased fecal egg counts (FEC) in all groups receiving anthelmintic treatment when compared to the untreated control group which proved the success of parasitic worm treatments. The parasite reduction outcomes differed between the treatment groups since drug mechanisms varied plus anthelmintic resistance could be present as well as there might have been discrepancies in dosing precision. The study results match previous research proving that combined parasitic medication strengthens control measures and postpones drug resistance development. These

methods cannot ensure persistent sustainability in the long run. The excessive use of multiple anthelmintic might develop into parasite populations that become resistant to multiple drugs thus diminishing their therapeutic value. Regular fecal egg count reduction tests (FECRT) together with targeted drug applications maintain drug effectiveness in the long term.

Present study showed that Nilzan Plus was best anthelmintic drug, followed by ivermectin and Albendazole was least effective. Another study aligns with these findings that for various gastrointestinal tract parasites, Nilzan was considered the most effective anthelmintic drug because it enhanced the pharmacokinetic properties and has long lasting effect on the digestive system of cattle ([Oliveira et al., 2023](#)). Another study highlighted that ivermectin also has a wide efficacy against nematodes ([Ahmed et al., 2017; 2021](#)). The post-treatment assessment showed FEC remained in various groups thus potentially indicating resistant parasite populations. Extensive studies suggested that Albendazole resistance (benzimidazole-based drugs) increased in cattle due to its frequent use ([Patel et al., 2022](#)). The observed lower efficacy in this study further supports concerns regarding drug resistance, necessitating the need for integrated parasite management strategies. Public health authorities need to maintain regular parasite monitoring and modify treatment methods to avoid resistance from becoming a serious widespread problem. Clinical outcomes of worm infections depend heavily on unique characteristics found within each farm according to the research results. Variables such as management styles and grazing systems and population density and environmental factors affected the parasite burden levels between farms. Rotational grazing practices together with sanitary improvements in farms led to lower FEC starting levels because integrated parasite management provides effective infection rate reduction ([Fernandez et al., 2023](#)). Broad parasite control approaches prove to be ineffective according to these results. Farm-specific parasite management plans should be developed to account for both regional environmental aspects and climate variations and soil conditions as well as pasture contamination levels. Better parasite control results can be achieved by developing treatment plans

which specifically target the conditions found on each individual farm.

The research findings showed that resistance development of anthelmintics stands as a major concern. Parasite burden reductions observed within the study were substantial, but residual FEC levels prove that some parasites are adapting to commonly used anthelmintic drugs. Current resistance patterns demonstrate the risk of conventional treatments becoming ineffective which would create more parasites and economic losses in livestock farms. Training programs that offer best practices regarding anthelmintic utilization should be provided to farmers alongside veterinarians. Monitoring drug dosages accurately and following withdrawal time requirements and moving between different medication classes prevents drug resistance from emerging. Proper proactive management of resistance is essential to maintain long-term anthelmintic effectiveness because it will otherwise demand new parasite control methods. Diarrhea-causing parasites affect dairy cattle badly because they decreased feeding effectiveness as well as weight reduction and diminished milk yield. Such decreases in fecal egg counts followed by treatment demonstrate better herd well-being that ultimately brings advantages such as greater profitability to dairy farming operations. Research has already shown that proper parasite management leads to improved production outputs, but this study specifically measured FEC reduction instead of production results. When parasites burden is reduced cows absorb nutrients better which allows them to gain weight efficiently and produce more milk. The improved health status makes supplementary veterinary care unnecessary thus generating extra economic value for farmers. Identifying the financial effects of anthelmintic treatments remains essential to determine the best treatment methods for dairy farms. When assessing cost-benefits in farming operations researchers need to analyze both medication costs along with veterinary fees and establish financial returns from increased milk output and reduced losses from diseases. Economic modeling allows farmers to assess the financial performance of various treatments through analytical methods which help farmers decide on interventions that enhance herds' productivity and health (Charlier et al., 2014). Thorough knowledge

about economical parasite control methods enables profitable long-term farm operation while preventing parasite resistance. Improved diagnostic methods help improve parasite management because they allow for better and more timely measurements of parasite infection levels. The traditional FEC testing works well but scientists have developed PCR and ELISA along with other molecular diagnostic tests to analyze resistant parasite strains with higher precision according to (Kaplan and Vidyashankar, 2012). Employment of diagnostic tools throughout standard herd management practices enables farmers to direct antiparasitic treatments toward specific cases thus minimizing medication waste and protecting against parasite resistance growth. The regular application of advanced diagnostic techniques for parasite population analysis supports the development of more effective control strategies.

## 5. CONCLUSION

It was concluded different anthelmintic treatments can effectively fight gastrointestinal parasites within dairy cows which promotes improved herd health and productivity as well as extended sustainability. It was noticed that the anthelmintic drugs successfully decreased the presence of Fecal Egg Counts which established their ability to combat parasitic infections. However, it was also suggested that the prevalence of parasites was also related to the implementation of hygienic practices and improvement in farm management practices can help to enhance animal health.

## 6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interest regarding the publication of this article.

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Table 1. Parasitic Burden in Pre-treatment Samples

Farms	Total Samples	Bovine Hook worm	Toxocara spp.	Moniezia spp	Rumen Fluke	<i>Haemonchus contortus</i>	Schistosoma spp.	Cooperia spp.	Tricho-strongylus spp.	<i>Ostertagia ostergai</i>
A	9	4	6	3	4	0	1	0	0	0
B	10	6	0	1	3	1	0	0	1	1
C	7	1	0	0	2	0	0	0	1	0
D	14	8	0	5	7	0	0	3	0	0

Table 2. Parasitic Prevalence and Total Egg Count in Pre-Treatment Samples of different Farms

Parasite	Total EPG (Pre-Treatment) Farm A	Total EPG (Pre-Treatment) Farm B	Total EPG (Pre-Treatment) Farm C	Total EPG (Pre-Treatment) Farm D
Bovine Hookworm	1350	3000	1000	450
Toxocara	1900		300	—
Moneizia	700	250	500	—
Rumen Fluke	1000	1200	500	1900
Haemonchus	700	550	—	400
Schistosoma spp.	250		—	—
Schizonts of Emiria	200		—	—
Cooperia	—	350	450	400
Ostertagia spp.	—	—	350	—

**Table 3. Parasitic Burden in Post-treatment Samples**

Sr. No.	Albendazole		Ivermectin		Nilzan Plus	
	FEC Reduction Rate % on day 7	FEC Reduction Rate % on day 14	FEC Reduction Rate % on day 7	FEC Reduction Rate % on day 14	FEC Reduction Rate % on day 7	FEC Reduction Rate % on day 14
1	50	100	81.82	100	88.89	100
2	30	70	90	100	84.21	100
3	42.8	71	58.33	100	70	90
4	50	100	57.14	71.43	57.14	100
5	33.3	77	60	100	60	100
6	50	100	-	-	50	100
7	45.45	100	-	-	85.71	100
<b>Mean ± SEM</b>	43.07±3.14	88.28±5.58	69.45±6.85	94.28±5.714	70.85±5.90	98.57±1.42

Table 4. Comparison of Pre-treatment Infection Rate of Each GIT Parasite in Normal, Semi-solid and Diarrheic Samples

Types of samples ➔ Parasite Name ↓	Normal Sample <i>n</i> = 24		Semi-solid Sample <i>n</i> = 11		Diarrheic Sample <i>n</i> = 5		Total Infection Rate (%)
	Positive Samples	Rate %	Positive Samples	Rate %	Positive Samples	Rate %	
<b>A. Nematodes (Roundworms)</b>							
1. <i>Bovine hookworm</i>	9	37.5	6	54.5	4	80	47.5(57.3)
2. <i>Toxocara spp.</i>	2	83(08)	3	27.27 (27)	2	40	17.5(25)
3. <i>Trichostrongylus spp.</i>	-	-	1	9	1	20	5(9.6)
4. <i>Cooperia spp.</i>	-	-	1	9	2	40	7.5(16.3)
5. <i>Ostertagia spp.</i>	-	-	1	9	-	-	2.5(03)
6. <i>Haemonchus spp.</i>	-	-	2	18.2	2	40	1(20.1)
<b>B. Trematodes (Flukes)</b>							
1. Rumen Fluke ( <i>Paramphistomum cervi</i> )	6	25	4	36.36 (36)	4	80	35(47)
<i>Schistosoma spp.</i>	-	-	1	9.09 (9)	-	-	3%
<b>C. Cestodes (Tape worms)</b>							
1. <i>Moneizia spp.</i>	4	16.6 (16)	2	18.18 (18)	1	20	18