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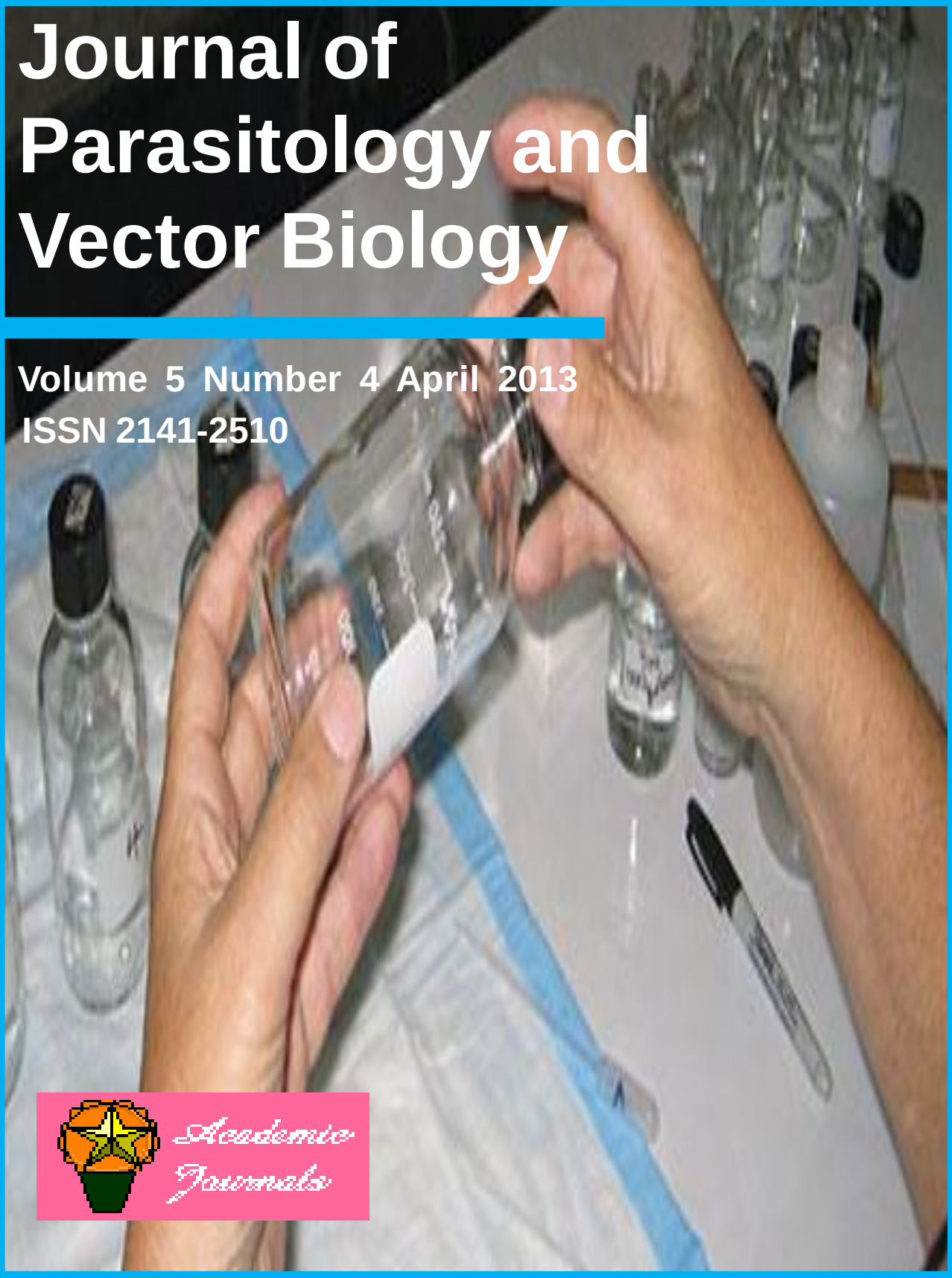

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Journal of Parasitology and Vector Biology

*Full Length Research Paper*

# Reuse of experimental huts for indoor residual spraying is feasible

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**Experimental huts are costly investments for programs evaluating insecticides and researching mosquito behaviour. These huts can be used to evaluate indoor residual sprays for malaria control but when the hut trials finish, contamination by the long lasting insecticide treatments might prevent further use of the hut. To see if experimental huts could be reused after indoor residual spraying tests, huts in Cotonou, Benin, were treated with a high dose of chlorpyrifos methyl (500 mg/m²). Bioassays with susceptible *Anopheles gambiae* indicated the treatment was successful. After this, untreated surfaces were washed and the inner surface of the cement walls was chipped away and replaced. Bioassays indicated that contamination was not present and that reuse of huts after indoor residual spraying is possible.**

**Key words:** *Anopheles gambiae,* experimental huts, reuse, indoor residual spraying, Benin, chlorpyrifos methyl.

## INTRODUCTION

Indoor Residual Spraying (IRS) has been an effective tool in the control of malaria vectors. In India, the number of cases of malaria was dramatically decreased, from 75,000,000 cases in 1947 to 100,000 cases in 1965, after 7 years of eradication operations, largely through IRS programs (Sharma, 1987). Significant reductions were also attributed to IRS programs in Kenya and Tanzania (Draper and Smith, 1962). The primary vector control intervention at the moment is insecticide treated nets (ITN), which are being widely distributed, especially for children and pregnant mothers. However, indoor residual spraying is also being rapidly scaled up; the number of people protected by IRS increased from 15 million in 2006 to 59 million in 2008 (WHO, 2009).

The World Health Organization (WHO) has provided guidelines for the testing of indoor residual sprays (WHO, 2006). These include three test phases. Phase I is composed of bioassays in the laboratory. Phase II trials are carried out in experimental huts, and Phase III evaluations are large-scale field trials. In addition to IRS treatments, evaluations of insecticides, repellents, and even behavioural studies can be tested in experimental huts (WHO, 2006; Suwonkerd et al., 2006).

Experimental huts are small houses with entrances allowing mosquitoes to enter but not easily leave. Thus all mosquitoes entering the hut can be counted and scored the following morning as alive or dead, and bloodfed or unfed. The addition of a veranda allows induced

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exophily to be measured as mosquitoes try to distance themselves from a treatment. Hougard et al. (2007) described the type of experimental hut used in this study.

As experimental huts are expensive to build and maintain, it is important to know whether or not the huts can be reused after treating the walls with a residual insecticide. Any contamination of the walls or other structures could result in misleading results in later tests. The WHO recommends a complete refurbishment of huts between trials to prevent this contamination (WHO, 2006). The feasibility of reusing huts by removing and replacing the inner surface of the walls was tested in Cotonou, Benin. Mortality of *An. gambiae* in cone bioassays was used as a measure of contamination.

## MATERIALS AND METHODS

Three experimental huts were selected for use in Ladji, a neighbourhood in Cotonou, Benin (6°23'23N, 2°25'56E). Ladji is on the edge of Lake Nokoué and has experimental huts that have been in use, primarily for testing insecticide treated mosquito nets, since 2002 (N'Guessan et al., 2010).

An initial series of bioassays was conducted to determine the mortality of mosquitoes exposed to various surfaces in the huts before treatment. Bioassays were performed in WHO cones attached to the surfaces with masking tape. Surfaces tested included doors (2 cones), walls (10 cones), entry window slits (2 cones), ceiling (2 cones), floors (2 cones), and verandas (2 cones). The locations of the cones were in random locations for the treated walls and very near the areas treated for untreated surfaces. Mosquitoes used were female *An. gambiae* s.s. Kisumu (a pyrethroid-susceptible laboratory strain originally from Kenya) between two and three days old. Five mosquitoes were put into each cone for 30 min. After this period, they were removed from the cone and put into plastic cups covered with untreated mosquito netting and given access to 10% honey solution. Mortality was scored after 24 h.

After the initial survey, two huts were treated with the organophosphate chlorpyrifos methyl ('Reldan GF 1246', Dow AgroSciences) at a dose of 500 mg/m$^2$. This was intended to represent a highly effective dose for indoor residual treatments for this insecticide. Ansari and Razdan (2004) came to the conclusion that an IRS treatment of 500 mg/m$^2$ gave high efficacy in controlling *Anopheles culicifacies*. N'Guessan et al. (2010) found chlorpyrifos methyl (500 mg/m$^2$) to provide high levels of control for a longer period than DDT (2 g/m$^2$) and the pyrethroid, lambda-cyhalothrin (30 mg/m$^2$). It is estimated that this dose would give a good idea if any of the insecticide was remaining after the resurfacing of the cement walls. The window slits that allow mosquitoes to enter, the metal door, the plastic ceiling, the floor near the wall, and the veranda were not treated and these surfaces were covered with plastic sheeting in one treatment hut (Hut 1), and with cement bag paper in the other (Hut 2). Reed mats were treated and nailed to the ceiling. Treatment was performed using a Vexmorel 2000 Pro (Berthoud, Villefranche, France) backpack sprayer. Five days after the treatment, bioassays were repeated following the same procedures.

After these bioassays, the interior structures of the huts, including the floors, were washed using laundry detergent (Omo; Unilever Nigeria, Aba, Nigeria). The surface of inner walls of the treated rooms (1 to 2 cm, not including the veranda) was chipped away by a mason. They were then resurfaced using a cement and sand mixture.

The walls were left to dry for four days. After this period, the original bioassays were repeated using *An. gambiae* (Kisumu) of the same age (2 to 3 days).

The numbers of dead mosquitoes compared to total numbers were analysed using blocked logistic regression, using Stata 8.1 (Stata Co., College Station, Texas, USA).

## RESULTS AND DISCUSSION

The mortality of mosquitoes on the different interior surfaces of the huts is shown in Table 1. Before the treatment, mortality was low in all huts, as expected. Huts 1 and 2 did not have any significant differences in overall mortality relative to the control hut ($p>0.05$).

All mosquitoes tested in the huts treated with chlorpyrifos methyl at 500 mg/m$^2$ died, whether they were exposed to treated surfaces or not. This was not expected as many of the surfaces tested had been covered during the treatment. It was unlikely that this contamination of untreated structures came from a faulty covering with the plastic sheeting or cement bags, as these were well secured. Mosquitoes that were held in cups in the room during testing but were not exposed to treated surfaces were also found dead after 24 h. This indicates there was probably another cause of the mortality of the mosquitoes than a direct contamination of these structures. Chlorpyrifos methyl has a low vapour pressure which may have contributed to the mortality of the mosquitoes, particularly as the tests were done only five days after the initial treatment. The huts were also closed after the treatments and not opened until the time of testing. A vapour effect in the veranda is worrying as it may result in an increased number of dead mosquitoes the verandas. Bar-Zeev and Self (1966) found greater mortality of mosquitoes in window traps on huts treated with propoxur and bromphos than in window traps on control huts, though in both cases the mosquitoes were not in contact with the treated surfaces indoors. As the main experimental hut models all use window traps or verandas to monitor mortality in exiting mosquitoes, this could lead to an overestimation of the insecticidal effect on mosquitoes that come into contact with treated walls and attempt to leave the hut.

There was little (less than 10%) mortality in mosquitoes tested on surfaces in the control hut was at the same time as the treated huts (shown in Table 1 as "after treatment").

After the removal of the inner surface of the walls and its replacement, the surfaces were tested again. There was some mortality (<15%) on the walls of Hut 2, and in the control, so these tests were repeated to see if this was mortality due to the treatment or not. In the end, no

**Table 1.** Results of WHO cone tests using *Anopheles gambiae* (Kisumu) in experimental huts in Ladji, Cotonou (Benin).

| Test condition | Percentage mortality (number tested) | | | | | |
|---|---|---|---|---|---|---|
| | Walls | Doors | Window slits | Floor | Ceiling | Veranda |
| **Hut 1 (plastic covering)** | | | | | | |
| Before treatment | 2.0 (49) | 0 (10) | 0 (10) | 10 (10) | - | 0 (9) |
| After treatment | 100 (50) | 100 (10) | 100 (10) | 100 (10) | 100 (9) | 100 (10) |
| After refection of walls | 1.0 (105) | 0 (23) | 0 (20) | 5.0 (20) | 0 (9) | 4.2 (24) |
| **Hut 2 (cement bag covering)** | | | | | | |
| Before treatment | 0 (53) | 0 (9) | 0 (8) | 12.5 (8) | 0 (4) | 11.1 (9) |
| After treatment | 100 (47) | 100 (10) | 100 (8) | 100 (10) | 100 (9) | 100 (10) |
| After refection of walls | 3.7 (215) | 5 (20) | 4.2 (24) | 4.2 (24) | 0 (11) | 4.3 (23) |
| **Control** | | | | | | |
| Before treatment | 0 (50) | 0 (8) | 0 (10) | 0 (7) | 0 (1) | 0 (9) |
| After treatment | 0 (46) | 0 (12) | 0 (11) | 9 (11) | 0 (10) | 0 (10) |
| After refection of walls | 1.9 (105) | 9.1 (33) | 4.5 (22) | 0 (24) | 4.8 (21) | 0 (22) |

hut had mortality greater than 10% on any surface. The results of mortality on covered structures for the hut with plastic coverings and the hut with cement bag coverings were not different before and after treatment (p=0.255). The plastic covering seemed to allow a better covering of surfaces to be left untreated. However, the run-off of the spray that happened to touch the plastic ran down the plastic sheeting more easily than on the cement bags, which absorbed some of the spray. It is important to keep this in mind while spraying to avoid having these drops touch surfaces that are not to be treated.

Before removing the inner layer of cement from the walls, the huts were vigorously cleaned. The plastic ceilings were not protected during spraying but were cleaned with soap and water. The fact that the ceilings caused no mortality in the final tests shows the impact of cleaning with soap and water.

## Conclusions

The results from the treated huts indicate that reuse of experimental huts is possible, as the results of all the tests before the treatment and after the refection were not significantly different. The most important steps in reuse of experimental huts are: good covering of structures to be protected, vigorous cleaning of these structures before the refection of the walls (to avoid contaminating the new walls), a complete removal of the first layer of cement, and a proper refection of walls. The findings from this study provide conclusive evidence that proper refection of huts allows for their reuse, even with highly insecticidal and long lasting products.

## ACKNOWLEDGEMENTS

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Journal of Parasitology and Vector Biology

*Full Length Research Paper*

# Prevalence of lungworm infection in small ruminants in North Gondar zone, Amhara National Regional State, Ethiopia

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**The study was conducted to determine the prevalence, associated risk factors and identification of the species of lungworms of small ruminants in four districts of Northwestern Amhara National Regional State. A total of 632 small ruminants were included in the study using coprologic procedures (565 animals) and post-mortem examination (87 animals). The study showed that the overall prevalence of lungworm infection was 46.0 and 56.3% based on coprological and postmortem examination, respectively. Significant difference (P < 0.05) in prevalence of lungworm was found between animals under semi-intensive and extensive management systems. The prevalence of lungworm infection was significantly different (P < 0.05) between seasons, the highest being during the rainy season (57.1%) and the lowest in the dry season months (35.0%). The prevalence of *Muelleris capillaris* infection was highest (50.4%) during the study period based on postmortem examination. The prevalence of lungworm infection showed significant association (P < 0.05) with different age groups of animals, where *Dictiocaulus fillaria* was highly prevalent in young age group while *M. capillaris* and *Protstrongylus rufescens* were highly prevalent in adult age groups of study animals. In the current study, three respiratory helminthes of small ruminants were identified and management system, age and season are important risk factors associated with lungworm infection in the study area. Due to its impact on production, emphasis should be given for the prevention and control of lungworm infection in the study area.**

**Key words:** *Dictiocaulus fillaria, Muelleris capillaries, Protostrongylus rufescens*, lungworm, small ruminant, helminthes.

## INTRODUCTION

Sheep and goats are the most numerous of man's domestic livestock and are especially important in the more extreme climates. Of the worlds' 1,614 million sheep and 475 million goats, 65 and 95%, respectively, are located in developing countries. Small ruminants in Africa are noted for their ability to convert low cost feed into high value products, namely: meat, milk, fiber, manure and hides (Wilsmore, 2006; Food and Agriculture Organization (FAO), 1986).

The current sheep and goat populations of Ethiopia are approximately 23 and 17 million, respectively (ILRL, 2000). Small ruminants are important contributors to food

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production in Ethiopia, providing 33% of meat consumption and 14% of milk consumption (Asfaw, 1997). In the central highlands of Ethiopia where mixed crop-livestock production system is practiced, small ruminants account for 40% of cash income and 19% of the house hold meat consumption (Zelalem and Fletcher, 1993). Owing to their high fertility, short generation interval and adaptation even in harsh environments, sheep and goats are considered as investments and insurance to provide income to purchase food during seasons of crop failure and to meet seasonal purchases such as improved seed, fertilizer and medicine for rural households (Asfaw, 1997).

Even though small ruminants are important components of the Ethiopian farming system, their contribution to food production, rural income and export revenue are far below than their expected potential. This is because small ruminant production is constrained by the compound effects of disease, poor feeding and poor management (Getachew, 1995). Studies in the central highland of Ethiopia have shown that lungworm parasites are a major problem in small ruminants and cause disease, increase mortality and production losses (Bekele et al., 1992). Up to half of all sheep deaths and morbidity on farms in Ethiopian highlands are caused by parasitic pneumonia and endoparasites (ILCA, 1990).

The incidence of lungworm infection varies geographically. Only a few limited studies have been completed on respiratory helminthes of small ruminants in the Amhara National Regional State, northwestern Ethiopia. Therefore, the aims of the present study are (i) to determine the prevalence of lungworm infection in small ruminants; (ii) to identify the species of lungworms and (iii) to identify associated risk factors for lungworm infection in north Gondar zone, Amhara National Regional State, northwestern Ethiopia.

## MATERIALS AND METHODS

### Study area

The study was conducted in four districts of northwest Amhara region including Abaintonios, Anchew, Belagig and Gondar town districts. The study areas are about 710 km North West of Addis Ababa at an altitude ranging from 1500 to 3500 meters above sea level. Numerous mountains, plateaus, sloped areas, rivers, streams and lakes mark the topography of the area. The climate of the study area is divided into subhumid ("wurch and Dega"), semiarid ("Woynadega") and arid ("Kola"). The average maximum and minimum daily temperature of the area varies between 22 to 30.7°C and 12.3°C, respectively.

The region receives a bimodal rainfall, the average annual precipitation rate being 1000 mm. The short rains occur during the months of March to May while the long rains extend from June to September. The production system observed in the area combines cereal-based agriculture and livestock farming. The study area has a livestock population of 2.03 million cattle, 0.6 million sheep, 0.54 million goats, 0.25 million equine species of which 62.5% are donkeys, 5% are mules, and 32.5% are horses, and 1.9 million poultry (CSA, 2003).

### Study animals and sampling

Small ruminants in the study area are kept mainly under an extensive traditional management system, involving small household flocks of mixed age. The sample size was determined according to Thrusfield (1995) for an infinite population. Since there has been no previous work done in the study area, an estimated prevalence of 50% was used for sample size determination. A total of 652 small ruminants (412 sheep and 153 goats from study districts, and 58 sheep and 29 goats from Gondar ELFORA abattoir) were used in this study. Four representative districts were purposely selected; Abaintoniose, Anchew, Blagig and Gondar town. Sampling animals was conducted at random from study districts and small ruminants slaughtered in ELFORA abattoir. Animals from Gondar town were managed under a semi-intensive system while the rest were under an extensive production system.

### Study design

#### *Coprological examination*

Faecal samples were collected directly from the rectum of all selected animals and stored in vials containing 10% formalin and transported to the Gondar veterinary clinic for laboratory examination. In the laboratory, the collected fecal samples were processed according to conventional methods for lungworm larvae (Baermanization) (Hansen and Perry, 1994). During sample collection, the species of the animal, sex, age, date of sampling and the area were recorded. In the laboratory, 25 g of faeces was weighed from each sample for the extraction of larvae using modified Baermann technique (Hansen and Perry, 1994). The faeces were enclosed in a gauze fixed on the string rod and submerged in a clean glass tube filled with warm water. The whole apparatus was left for 2 to 3 h. The larva leaves the faeces and migrates through the gauze and settles at the bottom of the glass. After siphoning off the supernatant, the sediment was examined under the low power of the microscope (Fraser, 1991; Urquhart et al., 1996). All the area under the cover slip was thoroughly and uniformly searched for the presence of lungworm larvae (Hendrix, 1998).

#### *Abattoir survey*

Small ruminants slaughtered at Gondar ELFORA abattoir were examined during the study period. The lungs were palpated for the presence of protostrogylidea nodules. These are brood nodules and worm nodules. Brood nodules are cone shaped granuloma-like areas of the affected lung tissue varying in size and color, which contains active worm and caused by parenchyma-dwelling small lungworms (Protostrongylidae). The worm nodules are pin head shaped mostly subpleural, grey (*Mullerius*) cyst which contain adult worms. If the nodules are present, they are trimmed off and worms extracted from the tissue by gentle pressing of a small non-calcified nodule or part of large nodule between two glass slides and then carefully teasing the worm away from the tissue. The air passages were opened starting from the trachea down to the small bronchi with fine blunt pointed scissors, to detect parasites (Kassai, 1999; Schneider, 2000).

### Data analysis

Prevalence was determined by the percentage (%) positive and chi-square ($x^2$). To measure association were

**Table 1.** Prevalence of lungworm infection in different districts of the study area.

| District | Number examined | Number positive (%) |
|---|---|---|
| Abaintonios | 176 | 77 (43.7) |
| Anchew | 88 | 50 (56.8) |
| Belagig | 56 | 24 (42.8) |
| Gondar town | 245 | 109 (44.4) |
| Total | 565 | 260 (46.0) |

P > 0.05.

the statistical tools applied and determined using statistical package for social sciences (SPSS) V 17.0. In all the analysis, confidence level was held at 95% and 5% level of significance (Thursfield, 1995).

## RESULT

### Coprological examination

Out of 565 (412 sheep and 153 goats) fecal sample examined, 260 (46.0%) were positive for lungworm infection. No statistically significant difference was observed in the prevalence of the lungworm infection of small ruminants in different district ($x^2$ = 28.22 P > 0.05). The higher prevalence rate was encountered in Anchew (56.8%) while the lowest was in Belagig (42.8%) (Table1).

The prevalence rate of lungworm infection was found to be higher in extensive management system (51.8%) than in semi-intensive management system (24.5%). Comparison between sheep and goats showed statically significant difference between the two species ($x^2$ = 33.2, P < 0.05). Similarly, there was association between the prevalence rate of lungworm infection with management system, and months where $x^2$ = 36.71, P < 0.0 and $x^2$ = 47.55 P < 0.05, respectively. The prevalence rate of lungworm infection did not show statistically significant association with sex of the animals ($x^2$ = 4.55, P > 0.05). Lungworm infection in small ruminants and associated risk factors in the study area are shown in Table 2.

In comparison of age groups, significant difference ($x^2$ = 33.22, P < 0.05) in the prevalence rate of lungworm infection was observed. Among lungworms identified, high prevalence rate of *Muelleris capillaries* (50.4%) followed by *Dictiocaulus fillaria* (26.5%) was recorded. The prevalence of *M. capillaris* and *Protostrongylus rufescens* increases with age of the animal, whereas the prevalence of *D. filaria* was high in young age groups of the animals. Mixed infection was common in old age group of the animals (Table 3).

### Abattoir survey

Of the 87 animals (58 sheep and 29 goats) examined in the ELFORA abattoir, 49 (56.3%) were positive for lungworm infection. There was a significant difference in prevalence of lung worn infection between the two species ($x^2$ = 3.95, P < 0.05), the prevalence being higher in sheep (63.8%) than goats (41.3%). The distribution of lungworm species detected in the lung during postmortem examination of positive animals is shown in Table 4. Higher prevalence of *M. capillaris* (51.0%) was observed than *P. rufescens* (28.6%) and *D. fillaria* (20.4%). The relative percentage of adult lungworms recovered during postmortem examination is described in Table 4.

## DISCUSSION

This study revealed the overall prevalence of lungworm infection of small ruminants to be 46.0%, with 42.3% in sheep and 46.7% in goats. It also disclosed that animals are infected with three nematode species parasitizing the respiratory tract of small ruminants of which *Mullerius* and *Dictyocaulus* species are the most abundant. This finding is in agreement with Regassa et al. (2010) and Sissay (1996) who have reported 40.4 and 44% in small ruminants in Dessie and Kombolcha districts, north-eastern Ethiopia and Bahir Dar, respectively. The present finding is lower than reports of Alemu et al. (2006) in northeast Ethiopia and Bekele and Abu (2011) in Tiyo District, South-East Ethiopia, with an observed prevalence of 53.6 and 57.1%, respectively. However, it is higher than that of Addis et al. (2011) and Weldesenebet and Mohamed (2012), with 33.83% prevalence at Gondar and 26.7% at Jimma, respectively. These differences might be due to the method used for the detection of the larvae or difference in the study area of topography which has a conducive environment for the survival of larvae and intermediate hosts, slug or snails and also the nutritional detection.

In this study, small ruminants under extensive management system had more infection than those kept under semi-intensive management system. The reason for this could be increased cultivation of land which restricts animals on communal grazing land so that large numbers of the animals are kept together. This could increase the degree of pasture contamination leading to higher prevalence rate. Management practice such as provision of ample nutrition increases the resistance of the host under the semi-intensive system, contrary to this malnutrition which reduces the host-parasite response and favors the fecundity of the parasites that allows the animals for continuous larvae exposure under extensive system (Soulsby, 1986).

The reason for this high prevalence of *Mullerius* compared with *Dictyocaulus* could be partly attributable to its wide range of intermediate host and the ability of larvae to over winter in the mulluscs. Additional factors which play a part in ensuring the endemicity of the worm are, first, the ability of L1 (First stage larva) to survive for months in faecal pellets and secondly, the persistence of

**Table 2.** Lungworm infection in small ruminants and associated risk factors in the study area.

| Variable | Number examined | Number positive (%) |
|---|---|---|
| **Species** | | |
| Ovine | 412 | 164(39.8) |
| Caprine | 153 | 96(62.7) |
| **Sex** | | |
| Female | 329 | 158(48.0) |
| Male | 236 | 102(43.2) |
| **Management system** | | |
| Extensive | 444 | 230(51.8) |
| Semi-intensive | 121 | 30(24.5) |
| **Season** | | |
| Dry | 285 | 100(35.0) |
| Rainy | 280 | 160(57.1) |

**Table 3.** Prevalence of the species of lungworm in small ruminants of different age groups.

| Age (years) | Prevalence (%) | Positive | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | Single infection | | | Mixed infection | | | | |
| | | D (%) | M (%) | P (%) | DM (%) | DP (%) | MP (%) | DMP (%) | |
| ≤1/2 | 29(11.6) | 14( 20.3) | 12(9.2) | 2(4.9) | - | - | 1(14.3) | - | 81 |
| 1/2-2 | 80(30.8) | 24(34.9) | 41(31.3) | 10(24.4) | 2(33.3) | 1(25.0) | 2(28.6) | - | 193 |
| 2-4 | 69.(26.5) | 12(17.4) | 41(31.3) | 11(26.8) | 3(50.0) | 1(25.0) | 1(14.3) | - | 146 |
| >4 | 82(31.5) | 19(27.4) | 37(28.2) | 18(43.9) | 1(16.7) | 2(50.0) | 3(42.9) | 2(100.0) | 145 |
| Total | 260(46.01) | 69(26.5) | 69(26.5) | 41(15.8) | 6(2.3) | 4(1.5) | 7(2.7) | 2(0.8) | 565 |

D:*Dictyocalus filaria*, M*: Muellerius capillaries*, P: *Protostrongylus rufescens;* $x^2 = 33.72$, $P < 0.05$.

**Table 4.** Relative percentage of adult lungworm recovered from postmortem examination.

| Species of lung worm | Number of positive | Percentage (%) |
|---|---|---|
| *D. filarial* | 10 | 20.4 |
| *M. capilaris* | 25 | 51.0 |
| *P. rufescens* | 14 | 28.6 |
| Total | 49 | 100.0 |

$x^2 = 87.0$, $P < 0.05$.

the L3 (third stage larva) in the intermediate host for the life time of the mollusks (Taylor et al., 2007). On the other hand, the longevity and development of free larvae of *Dictyocaulus* are known to be dependent on humidity and temperature condition. Dry seasons are characterized by high mortality of larvae in the pasture (Gallia and Nunns, 1976), where dry, hot summer and cold winter is the climatic condition of the study area. The prevalence of *D. filaria* is higher in infants than adults. However, the prevalence of *M. capillaris* and *P. rufescens* is higher in adult animals than younger ones. This might be due to the long period of potency and the apparent inability of the final host to develop acquired immunity, so that adult sheep have the heaviest infection, highest infection and the highest prevalence (Taylor et al., 2007). The study further revealed that goats were found to be more susceptible to lungworm infection than sheep. This could be because most of the goats in this study were from lowland and mid altitude areas, which are thought to be suitable for survival of the larval stage of the parasite. Likewise, in the lowland areas of the country where goats are mostly reared, there is poor veterinary infrastructure and medication.

More importantly, the condition could be due to less or slow development of immunity in goats compared to sheep (Wilsmore, 2006).

The higher prevalence of lungworm infection during the rainy season of the months might be the presence of sufficient moisture during the rainy season which favored the survival of infective larvae and stimulated the activity of mollusks in the pasture resulting in higher probability of up take of the infective larvae, leading to higher prevalence rate. In northwestern part of Ethiopia, under local production system, the animals that are completely managed on pasture grazing throughout the year succumb to seasonal variation of availability of forgeable feed and then difference in plane of nutrition. Thus, the presence of sufficient feed in rainy season could in turn increase the nutritional status, and these well fed animals develop good immunity that suppressed the fecundity of the parasites. This situation was reported by Bisset et al. (1996) that increased plane of nutrition increased the immunity and reduced the fecundity of the worm, contrary to the higher prevalence rate observed during the rainy season.

The prevalence of lungworm infection obtained by postmortem examination was higher (56.3%) than the result obtained by coproscopic examination (46.0%). This difference might be related to worm nodes of Protostronglidae. Worm nodules contain immature parasite in general (Schneider, 2000). Higher infection rate observed on postmortem examination may be related to these worm nodules detected. In addition, in the prepatent or postpatent phase or during hypobiosis, it is impossible to detect this parasite by fecal examination (Fraser, 1991). Furthermore, egg laying adult female parasites might be inhibited by the immune reaction of the host (Hansen and Perry, 1994).

## Conclusion

The study revealed that lungworm infection in small ruminant is highly prevalent in North Gondar zone. *D. filaria, M. capillaris* and *P. rufescens* were the lungworms identified during the study. Young animals are mostly affected by most pathogenic species of lungworm, *D. filarial*, than adults on the contrary which are highly affected by *M. capillaris* and *P. rufacens*. Animals under semi-intensive management system have been found less affected than those under extensive management system. Therefore, avoidance of overstocking and grazing in watery and damp areas of arid pastures, treatment of potential worm carriers and separate grazing of young stocks are recommended.

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# Two avian cestodes parasitic to *Corvus* species of Kashmir, India

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**Three species of birds belonging to the genus *Corvus* (*Corvus monedula*, *Corvus splendens* and *Corvus macrorhynchos*) were collected from nine different localities of Kashmir valley and investigated for the presence of helminthes. *Dilepis undula* (Schrank, 1788) was recovered from all the three host species. While, *Mayhewia kavini* Chishti and Khan, 1982 was recovered only from *C. monedula* and no specimen of this cestode was obtained from *C. splendens* and *C. macrorhynchos* during the present study. The specimens were identified and redescribed on the basis of various morphological and morphometric characters when compared to the known species of genera as *Dilepis* and *Mayhewia*, respectively. However, some intraspecific variations were observed when the present measurements were compared with those of previous authors. The prevalence, mean intensity and abundance of the parasites were determined.**

**Key words:** Cestode, crows, *Corvus*, *Dilepis*, *Mayhewia,* prevalence, Kashmir.

## INTRODUCTION

The present study was a part of helminthological investigation carried out on three *Corvus* species (*Corvus monedula*, *Corvus splendens* and *Corvus macrorhynchos*) of Kashmir from November, 2007 to May, 2009. *C. monedula* Linnaeus, 1758 (Jackdaw) is a black-plumaged passerine bird with distinctive white irises; sexes and ages are alike (Goodwin, 1983). It is omnivorous and feeds on plant material and invertebrates (Lockie, 1956). *C. splendens* Vieillot, 1817 (House crow) is about 40 cm in length with lighter grey-brown neck and breast. The wings, tail and legs are black. It appears to be associated with humans and no populations are known to exist independently of humans (Nyari et al., 2006). *C. macrorhynchos* Wagler, 1827 (Jungle crow) is a Large-billed crow with black glossy wings, tail, face and cause diseases include viruses, bacteria, protozoans and throat.The groups of parasites which infect birds and helminthes besides some arthropod ectoparasites. Sexually mature tapeworms live in the intestine or the diverticula of all classes of vertebrates (Roberts and Janovy, 2005). These parasites are found more frequently in warmer seasons, when intermediate hosts are abundant. Birds become infected by eating an intermediate host (infested with larval stage of a cestde), which may be an insect, crustacean, earthworm, slug, snail or leech depending upon the species of tapeworm (Calnek, 1997). Over 4,000 species of cestodes have been described from animals (Schmidt, 1986). Recently many workers have contributed to the knowledge of avian cestodes such as evaluation of genetic basis of host specificity of cestodes (Benesh, 2010; Henrich et al., 2013), description of ultrastructural characters of the spermiogenesis and mature spermatozoon of *Notopentorchis* sp. by means of transmission electron microscopy (Yoneva et al., 2012), description of some new cestode species like *Spiniglans sharpiloi* from *Pica pica* (Kornyushin et al. 2009), *Cotugnia orientalis* from *Gallus gallus domesticus* (Nanware et al., 2011).

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**Table 1.** Prevalence, mean intensity and abundance of *Dilepis undula* in *Corvus* species of Kashmir.

| Host spp. | NE | NI | NP | P% | MI | AB |
|---|---|---|---|---|---|---|
| *C. monedula* | 30 | 6 | 18 | 20 | 3 | 0.6 |
| *C. splendens* | 23 | 3 | 11 | 13.04 | 3.66 | o.47 |
| *C. macrorhynchos* | 12 | 3 | 11 | 25 | 3.66 | 0.92 |
| Total | 65 | 12 | 40 | 18.46 | 3.33 | 0.61 |

NE = number examined; NI = number infected; NP = number of parasites; P = prevalence; MI = mean intensity; Ab = Abundance.

**Table 2.** Prevalence, mean intensity and abundance of *Mayhewia kavini* in *Corvus* species of Kashmir.

| Host spp. | NE | NI | NP | P% | MI | AB |
|---|---|---|---|---|---|---|
| *C. monedula* | 30 | 4 | 10 | 13.3 | 2.5 | 0.33 |
| *C. splendens* | 23 | 0 | 0 | 0 | 0 | 0 |
| *C. macrorhynchos* | 12 | 0 | 0 | 0 | 0 | 0 |
| Total | 65 | 4 | 10 | 6.15 | 2.5 | 0.15 |

NE = number examined; NI = number infected; NP = number of parasites; P = prevalence; MI = mean intensity; Ab = abundance.

However, systematic knowledge of cestodes from birds of Kashmir including *Corvus*, is still represented through a few references as is obvious by tracing the historical review of cestodes from aves of Kashmir (Gupta, 1967; Fotedar et al., 1970; Chishti, 1973, 1980a, b, c; 1981; Fotedar and Chishti, 1973, 1974, 1976a, b; 1977; Chishti and Khan, 1978; 1979; 1982; Chishti et al., 1986). *Choanotaenia infundibulum*, *Choanotaenia micracantha*, *Anomotaenia galbulae* and *Mayhewia kavini* are the only cestodes reported from *Corvus* species in Kashmir so far (Foterdar and Chishti, 1974; 1976; Chishti and Khan, 1982; Chishti et al., 1986). Thus a thorough investigation of helminth parasitism of *Corvus* species of Kashmir was imperative. The present paper gives redescription of two cestodes (*Dilepis undula* and *M. kavini*) collected from the intestines of three species of crows (*C. monedula*, *C. splendens and C. macrorhynchos*) caught from different localities in Kashmir.

## MATERIALS AND METHODS

### Study area

Kashmir valley is a temperate, North-west Himalayan region of Jammu and Kashmir states in India. It lies between 33° 20' and 34° 54' N latitudes and 73° 55' and 75° 35' E longitudes, covering an area of about 15,948 km$^2$. It is a deep bowl shaped valley bounded by lofty mountains of the Pir Panjal and the great Himalayan ranges. The floristic and faunal diversity of the valley is considerably rich owing to its unique topography, temperate climate and geographical isolation from the surrounding plains (Dar et al., 2002). The birds were collected from different localities of Kashmir valley like Barzulla, Bugam, Chadoora, Khansahib, Naseembagh, Rajbagh, Rambagh, Wathora, and Yousmarg. These collection sites lie within the radius of five to fifty Kilometers (km) from the center of Srinagar city, the summer capital of Jammu and Kashmir States in India and are easily accessible by road transport.

### Collection and processing of cestodes

During the present study, 65 birds belonging to three species of *Corvus* (*C. monedula* Linnaeus, 1758; *C. splendens* Vieillot, 1817 and *C. macrorhynchos* Wagler, 1827) were caught alive with the help of nylon net traps, locally known as "*Walwash*" using suitable baits. The hosts were slaughtered and dissected for parasitological investigation, and the cestode parasites thus collected were fixed in Carnoy's fixative, stained in acetoalum carmine and transferred to xylene for clearing before mounting them in dextrine plasticised xylene (DPX) (Meyer and Olsen, 1975). The drawings of the specimens were made with the help of prism type camera Lucida. Measurements were taken with objective and stage micrometers and expressed in mm (unless otherwise stated). The specimens were identified on the basis of various taxonomic characters using Yamaguti (1961), Schmidt (1986) and Chishti and Khan (1982). Photomicrography was conducted with the help of Digital Olympus Camera.

## RESULTS

Two cestode species (*D. undula* and *Mayhewia kavini*) recovered from the intestines of three species of crows (*C. monedula*, *C. splendens and C. macrorhynchos*) are

**Table 3.** Comparative measurements of *Dilepis undula* (Schrank, 1788) with present form (measurements in microns = µ).

| Characteristic | Davies (1935) | Mattrick (1958) | Chishti (1974) | Present author |
|---|---|---|---|---|
| No. of rostellar hooks | 45-60 | 48-62 | 64-70 | 46-58 |
| Size of outer rosterllar hooks (µ) | 84 | 91-116 | 72-80 | 74-85 |
| Size of inner rostellar hooks (µ) | 72 | 70-88 | 60-64 | 67-78 |
| No. of testes | 28-35 | 28-36 | 24-26 | 24-32 |

redescribed based on their detailed morphological and morphometric studies, revealing some minor intraspecific variations. The prevalence, mean intensity and abundance of each parasite were recorded (Tables 1 and 2).

### *Dilepis undula* (Schrank, 1788)

Hosts: *C. monedula, C. splendens* and *C. macrorhynchos*;
Locality: Naseembagh, Rajbagh, Wathora and Yousmarg;
Location: Intestine.

The following redescription is based on twelve cestode specimens, taking four specimens from each *Corvus* species. The cestodes measure 38 to 43 mm in length and 1.36 mm in maximum breadth. All the proglottids are broader and longer, and the posterior margin is broader than anterior margin. The mature proglottids measure 0.18 to 0.27 mm in length and 1.16 to 1.28 mm in breadth. The gravid proglottids measure 0.16 to 0.23 × 0.8 to 1.25 mm in length and 1.12 to 1.25 mm in breadth (Table 3 and Figure 1: A to E).

The scolex is globular and measures 0.44 to 0.54 mm in length and 0.60 to 0.64 mm in breadth across the suckers. The four suckers are rounded, muscular and measure 0.16 to 0.20 mm in diameter. The rostellum with its sac extends below the posterior margin of suckers and measures 0.44 to 0.58 mm in length and 0.14 to 0.20 mm in maximum breadth; it bears 46 to 58 hooks arranged in a double crown; each crown alternating with the other. The blade of hooks is smaller than handle, with guard as knob-like structure. The hooks of anterior and posterior crown measure 74 to 85 µ and 67 to 78 µ in length, respectively. The scolex is followed by a short neck that measures 0.24 to 0.33 mm in length and 0.50 to 0.55 mm in breadth. The dorsal longitudinal excretory duct is 14 µ in diameter and the ventral duct is 17 µ in diameter. The testes lie posterior to female reproductive organs, numbering 24 to 32 in each proglottid. They are rounded and measure 48 to 53 µ in diameter. The vas-deferens forms many coils in the anterior region of the proglottid. The vesicula seminalis are absent and the ductus ejaculatorius is straight. The cirrus pouch is cortical, extending up to the longitudinal excretory ducts of poral side; measuring 0.12 to 0.15 mm in length and 0.04 to 0.07 mm in width. The cirrus is slender and small 82 to 88 µ in length, armed with small spines. The genital pores are unilateral and present just near the anterior margin of proglottids, laterally.

The ovary is bilobed, with the poral lobe being smaller than the aporal one; the two lobes show further lobulations. The two lobes are connected in the middle by a narrow U-shaped isthmus. The ovary extends 0.65 to 0.72 mm across. The vitelline gland is compact, horse-shoe shaped and lies posterior to the ovary. It measures 0.12 to 0.17 × 0.04 to 0.06 mm. An oval receptaculum seminis, 0.08 to 0.10 × 0.03 to 0.05 mm in size, lies dorsal and anterior to the ovary. The uterus occupies the whole of the gravid proglottid filled with eggs, which measure 23 to 26 × 14 to 17 µ.

### *Mayhewia kavini* (Chishti and Khan, 1982)

Hosts: *Corvus monedula*;
Locality: Barzulla, Naseembagh, Rambagh and Wathora;
Location: Intestine.

The following redescription is based on ten cestode specimens, collected from the intestines of four, out of thirty jackdaws, *C. monedula.* The cestodes measures 55 to 84 mm in length and 0.77 to 1.0 mm in their maximum breadth. The strobila consists of many proglottids which are broader than being long. The mature proglottids measure 0.20 to 0.26 mm in length and 0.8 to 0.92 mm in breadth. The gravid proglottids are 0.30 to 0.36 mm in length and 0.77 to 1.0 mm in breadth (Table 4 and Figure 2: A to D).

The scolex is small and globular, measuring 0.15 to 0.18 mm, with everted rostellum up to the base of suckers, and 0.12 to 0.14 mm without rostellum. It measures 0.22 to 0.23 mm across suckers. The rostellum with its sac measures 0.17 to 0.21 mm in length and extends below the posterior margin of suckers. The rostellum bears a single crown of 10 wrench-shaped hooks which measure 26 to 28 µ in length. The handle of hook is larger than the blade and is parallel to the equally long blade. The suckers are rounded and muscular, measuring 0.06 to 0.08 mm in diameter. The scolex is followed by a short neck that measures 0.17 to 0.22 mm in length and 0.10 to 0.15 mm in breadth. The dorsal longitudinal excretory duct is 10 to 13 µ and ventral excretory duct is 16 to 20 µ in diameter.

Testes are three in number, disposed in a triangle, two being aporal and one poral. They measure 92 to 100 µ in diameter. Cirrus puch extends into the medullary

**Table 4.** Comparative measurements of *Mayhewia kavini* Chishti and Khan, 1982 with present form. (measurements in mm unless stated otherwise).

| Particulars | Chishti and Khan, 1982 *Mayhewia kavini* | Present author |
|---|---|---|
| Max. length | 60-90 | 55-84 |
| Max. breadth | 0.72-0.785 | 0.77-1.0 |
| Mature proglottids | 0. 22-0.25 × 0.65-0.72 | 0.20-0.26 × 0.8-0.92 |
| Gravid proglottids | 0.26-0.35 × 0.72-0.785 | 0.3-0.36 × 0.77-1.0 |
| Scolex length upto rostellum | 0.19-0.198 | 0.15-0.18 |
| Scolex length without rostellum | 0.138-0.146 | 0.12-0.14 |
| Width across suckers | 0.242-0.249 | 0.22-0.23 |
| Rosetellum length | 0.222-0.228 | 0.17-0.21 |
| Number of rostellar hooks | 10 | 10 |
| Hook size | 25-30 µ | 26-28 µ |
| Sucker size | 0.09-0.1 × 0.075-0.082 | 0.06-0.08 (Dia.) |
| Dorsal excretory duct | 10-12 µ | 10-13 µ |
| Ventral excretory duct | 18-22 µ | 16-20 µ |
| Testis diameter | 72-84 µ | 93-100 µ |
| Cirrus pouch extent | Medullary | Medullary |
| Cirrus pouch size | 0.14-0.155 × 0.052-0.057 | 0.16-0.19 × 0.050-0.053 |
| External vesicula seminalic | 0.16-0.18 × 0.06-0.072 | 0.18-0.25 × 0.07-0.072 |
| Internal vesicula seminalis | 0.06-0.072 × 0.048-0.052 | 0.05-0.065 × 0.42-0.50 |
| Genital pore position | Anterior 1/3 rd. | Anterior 1/3 rd. |
| Ovary extend across | 0.172-0.23 × 0.052-0.09 | 0.25-0.3 × 0.06-0.08 |
| Vitelline gland | 50-60 µ × 40-50 µ | 65-72 µ × 48-53 µ |
| Receptaculum seminis | 0.121-0.15 × 0.088-0.11 | 0.14-0.16 × 0.07-0.08 |
| Egg size | 42-54 µ × 30-41 µ | 38-44 µ × 28-33 µ |
| Embryo size | 28-32 µ × 18-22 µ | 22-26 µ × 16-20 µ |
| Embryonic hook size | 12-15 µ | 9-12 µ |

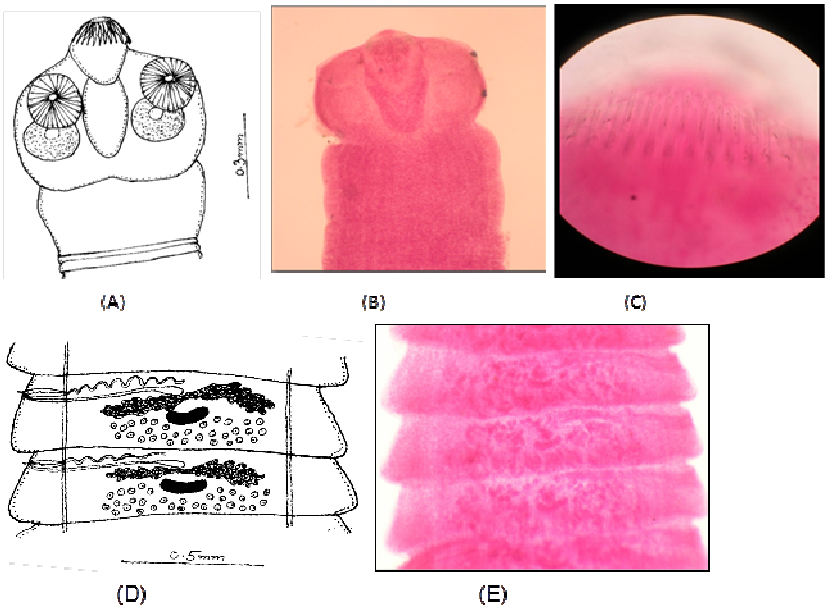


**Figure 1.** *Dilepis undula* Schrank, 1788. (A, B) Scolex showing suckers and rostellum with hooks; (C) rostellum showing hooks; (D, E) mature proglottids showing reproductive organs.

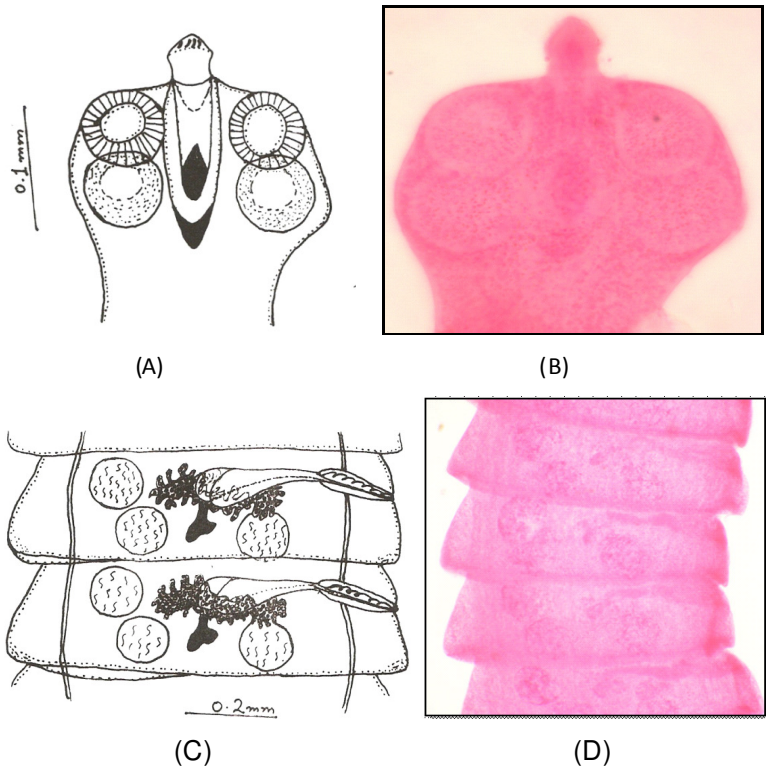


**Figure 2.** *Mayhewia Kavini* Chishti and Khan, 1982. (A, B) Scolex showing suckers and rostellum with hooks; (C, D) mature proglottids showing reproductive organs.

region and measures 0.16 to 0.19 mm in length and 0.05 to 0.053 mm in width. The external vesicula seminalis is large, extending to the middle of progrottids and measures 0.18 to 0.25 mm in length and 0.07 to 0.072 mm in width. The small and oval internal vesicula seminalis measures 0.05 to 0.065 mm × 0.042 to 0.05 mm. The genital pores are unilateral, opening in the anterior third margin of proglottid laterally. The follicular ovary lies in the middle of proglottid and is bilobed. It measures 0.25 to 0.3 mm across and 0.06 to 0.08 mm in thickness. Vitelline gland is slightly lobed and lies posterior to the ovary. It measures 65 to 72 × 48 to 53 µ. The receptaculum-seminis is flask-shaped, situated anterior to ovary and measures 0.14 to 0.16 × 0.07 to 0.08 mm. Receptaculum seminis narrows into a slender vaginal tube which opens into the genital atrium just posterior to the opening of cirrus pouch. The gravid proglottids are compactly filled with eggs which are oval in shape and measure 38 to 44 × 28 to 33 µ.The embryo measures 22 to 26 × 16 to 20 µ, and embryonic hooks measure 9 to 12 µ in length. In addition to *D. undula* and *M. kavini* redescribed in this paper, concurrent infestation with other helminth parasites such as *Echinostoma revolutum, Capillaria anatis, Anomotaenia galbulae* and *Choanotaenia micracantha* was also observed in some of the bird hosts during the present investigation.

## DISCUSSION

The distinguishing characteristics of the present *Dilepis* specimens are: 46 to 58 rostellar hooks arranged in double crown; that of the anterior and posterior crown measure 74 to 85 and 67 to 78 µ, respectively; testes 24 to 32 in number, postovarian; cortical position of cirrus pouch; large bilobed ovary and unilateral genital pores.

When compared with the known species of genus *Dilepis* Weinland, 1858 described from different avian hosts, the present specimens shows a large similarity of characters with *D. undula* (Schrank, 1788). This species has also been redescribed several times, giving variation in the number and size of rostellar hooks, number of testes and length of strobila (Table 1). However, the present measurements come in the range of those described by various authors. In view of these measurements and other similarities as described above, the present cestode specimens are assigned to *D. undula* (Schrank, 1788). It is recorded for the first time in *Corvus* species from Kashmir. The earliest record of this species in Kashmir is from *Sturnus vulgaris humii* and *Turdus unicolor* (Chishti, 1974). The present cestode specimens of genus *Mayhewia* collected from *C. monedula* possess characteristics like rostellum with single crown of 10 small hooks; strobila comprising of numerous transversely

elongated progrottids; two pair of excretory stems; three testes arranged in a triangle; cirrus pouch extending into medullary region; external and internal seminal vesicles present; ovary bilobed and uterus succular. All these characters are in conformity with the description of genus *Mayhewia* Yamuguti, 1956.

While comparing with the known species of genus *Mayhewia* Yamaguti, 1956, the present form shows a similarity of characters with those of *Mayhewia kavini* Chishti and Khan, 1982 as regards the number (10) and size of rostellar hooks, number of testes, extent of cirrus pouch, presence of seminal vesicles etc. However, some intraspecific variations were recorded in different structures as indicated in Table 2, the reason for which could be the age of parasite, host species, intensity of infection (higher intensity, smaller parasites), methodology (fixation), environmental factors of the study area, body conditions of the host, etc. (Ternopolskaya, 1984; Kuchai et al., 2012). Since these variations are of minor significance, therefore the present specimens in view of above similarities, are assigned to *Mayhewia kavini* Chishti and Khan, 1982.

## Conclusion

The present paper redescribes only two species of cestode parasites (*D. undula* and *M. kavini*) recovered from three species of the genus *Corvus*. This work adds to the parasite species diversity infecting these avian hosts and may prove helpful for the future research on helminthes of birds. In addition, the present study showed that *M. kavini* was recovered only from *C. monedula*, hence the parasite may be host specific in nature.

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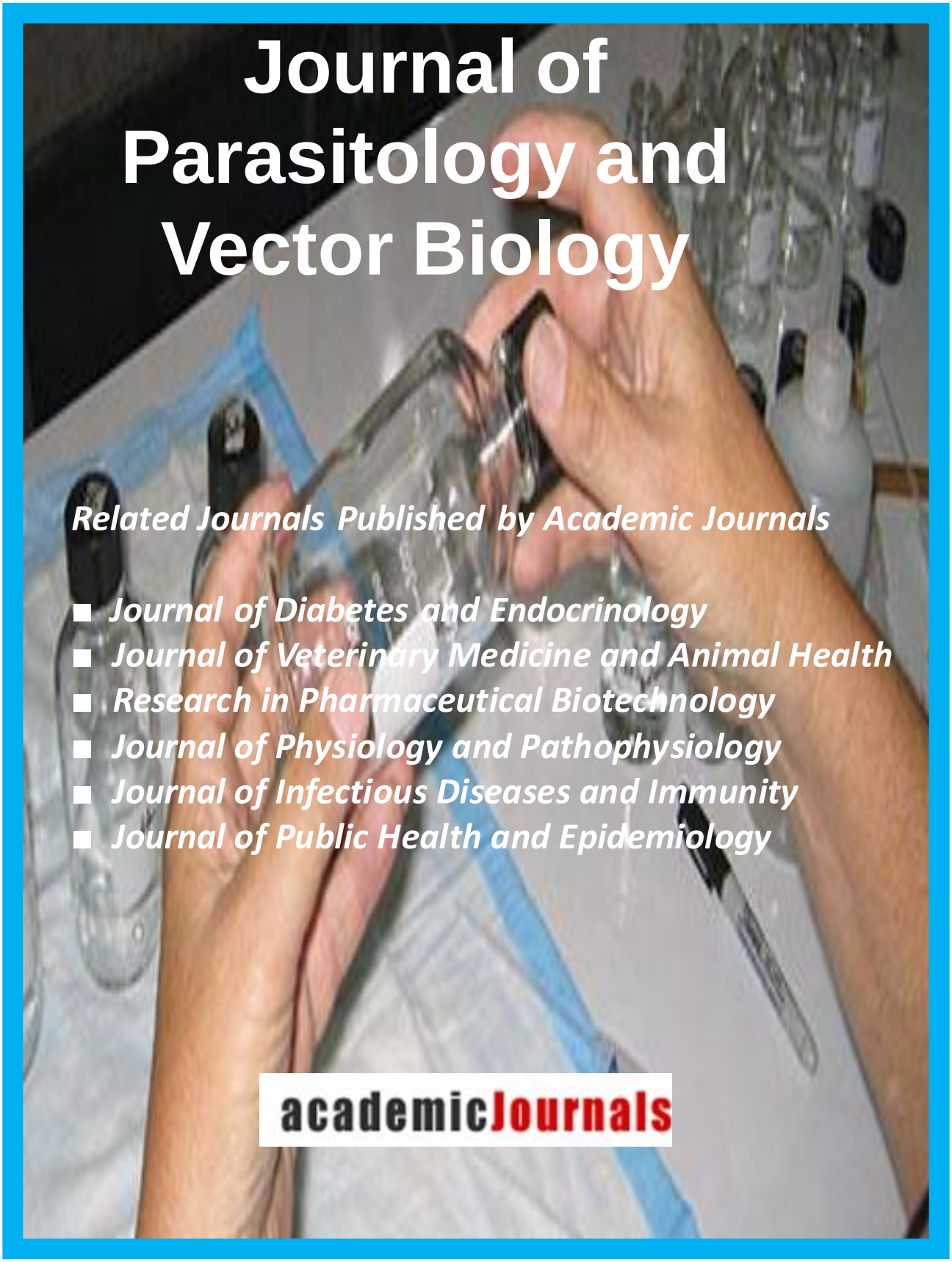

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