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## Histopathological studies of tapeworm *Raillietina tetragona* (Molin, 1858) from the gastro-intestine of indigenous chicken (*Gallus domesticus* L.) farming in Kirtipur, Nepal

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### Abstract

**Aim:** *Raillietina tetragona* is a parasitic tapeworm belonging to the class Cestoda and found in the small intestine of chicken throughout the world. Thus a research was undertaken into *Raillietina tetragona* infection in scavenging indigenous chicken *Gallus domesticus* in the Kirtipur Municipality of Kathmandu Valley from March 2013 to April 2015.

**Materials and Methods:** A total of 225 birds of different age groups and both sexes were randomly selected from 10 different farms and screened through clinical, parasitological and pathoanatomical examinations. Photomicrography was conducted with DP-12 Digital Camera attached to Olympus Research Microscope in the department of Zoology.

**Results:** The Cestode *R. tetragona* recorded during the different seasons (spring, summer, autumn and winter) and it was highest 19.64% parasites were recorded during autumn season while the infestations were as 18.37, 9.59 and 8.51 in the seasons of summer winter and spring respectively. Physiological status, bird type, production purpose, farm age (years), level of 19.64% biosecurity, and frequency of cleaning the pen were the risk factors significantly ( $p < 0.05$ ) associated with the presence of helminth infections.

**Conclusion:** This study shows that helminth infections are endemic in the study area, as 19.64% of the sampled farms were infected with *R. tetragona* species. Findings from this study provide information that will assist in improving the poultry sector in Kathmandu in general, for better production and profitability.

**Keywords:** Histology, gastrointestinal cestodes, Kathmandu, *R. tetragona*, poultry.

### 1. Introduction

Nepal is land-linked between two most populous countries of the world, India in the East, South and West, and China in the North <sup>[1]</sup>. Agriculture is the major sector of Nepalese economy and livestock, including poultry is an integral part of agricultural production system in Nepal, providing high value animal protein such as meat, milk and eggs for the human consumption. Contribution of agricultural sector in Gross Domestic product (GDP) of Nepal is 33% <sup>[2]</sup>. Among them contribution of livestock sector in total GDP is 26.8% and poultry sub sector within livestock contributes 8% of Agriculture Gross Domestic product (AGDP) and Indigenous poultry is widely prevalent which contributes 55% of total poultry population <sup>[3,4]</sup>. Farmers with a small land holding of between 0.2 and 0.5 hectares land keep almost 25% of the livestock. A distinct pivoted role of the livestock is well established in the farming system of Nepal since it functions as a supplier of mature and high value animal protein. Birds are important for their commercial, recreational, ethical, spiritual values and form a rich protein source for humans. There is a rich diversity of birds in this area. Among birds, poultry is of great importance in rural production system in this area. Chicken was the only poultry bird being reared for meat and egg production. During summer months, hens laid as many as 20 eggs/month whereas no egg production was reported during winter months; which may be attributed to the short day length coupled with very little scavenging feed resources. The domestic fowl and eggs provide an important source of protein for human consumption. The increased mortality and decreased productivity in chickens is mainly due to mismanagement,

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lack of nutritional feeding, diseases and predation [3]. Kathmandu Valley (KV) is the urban center of Nepal and includes five major cities: Kathmandu, Lalitpur, Bhaktapur, Kirtipur, and Thimi. Kirtipur is located about 6 kms south-west of the centre of Nepal's capital Kathmandu. While the old historical part of the village extends only over little more

than 1000m from west to east and hardly more than 250 m from north to south the entire Kirtipur Municipality with its 19 single villages (wards) covers nearly 15 square kms. The traveller coming from Tribhuvan International Airport or from the centre of Kathmandu reaches Kirtipur after a 20-30 minutes ride by taxi or by public bus.



**Fig 1:** Village area of Kirtipur Nagar Palika where local people farming domestic chicken *Gallus domesticus*

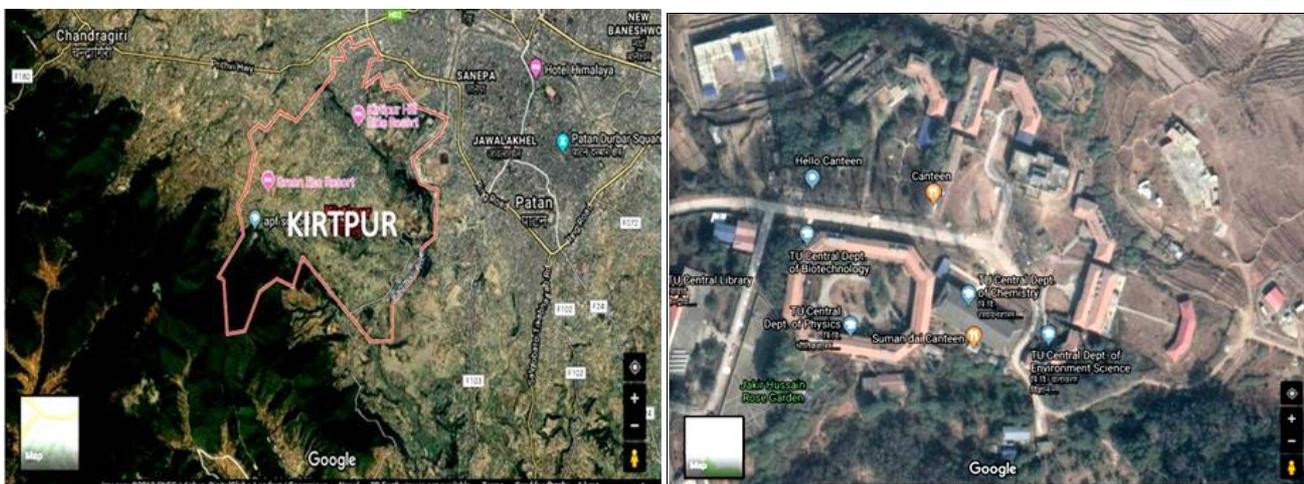
Poultry farming (Figure 1) in Nepal and its popularity increasing between local farmers they raising chickens organically for meats or eggs that provides a rapid return on your investment. In the poultry farms follow sustainable free range poultry or raising domesticated birds like chicken, turkey, emu, ostrich, guinea fowl, duck, goose etc. In Nepal, poultry farming is one of the commercial branch of agriculture practices. It is the main source of livelihood both in the rural and urban farming communities. The egg is also rich in vitamins like A, D, B1, B2 and pantothenic acid. The chicken meat contains a high quality of thiamine, riboflavin and pantothenic acid [4]. In Nepal, birds are important for their commercial, recreational, ethical, spiritual values and form a rich protein source for humans and poultry farming is fast growing meats and eggs business in Nepal and provides a rapid return on the investment [5]. Domestic birds have significant contributory role in national economy and social economic condition especially in rural areas of Nepal [2]. Farmers and agriculturists are still depending economically on these animals [3]. *Raillietina tetragona* belong to cestode causes cestodiasis in animals including domestic fowl (*Gallus domesticus*). The cestode inhabits the small intestine and causes stunted growth of young chicken, emaciation of the adult, and decreased egg production of the hen [6]. The resultant situation leads to loss of body weight, retarded growth, reduced egg production, weakened body resistance

and even death of the host [7]. In *R. tetragona* infection, the intestinal wall of the host intestine is thrown into ridges of purplish colour and the intestinal mucosa sloughs off. Thus, the present study was undertaken to find out gastrointestinal parasites and their histopathological studies in domestic Chicken (*Gallus domesticus* L.) collected from the Kirtipur of Kathmandu, Nepal.

## 2. Materials and Methods

### 2.1 Study area

Kathmandu is the capital of Nepal and is located in the central part of the country [8]. KV is of strategic importance as it is centrally located between China and India, and its urban settlements of Kathmandu, Lalitpur, Bhaktapur and Kirtipur became early trade centers [23]. These settlements continued as economically and politically important towns for hundreds of years [24]. Because of its livelihood options, KV is one of the most popular destinations for migrants from different parts of Nepal [9]. KV covers an area of 569.80 km<sup>2</sup> and includes three districts: Kathmandu, Bhaktapur, and Lalitpur [10] and Kirtipur Municipality lies in western side is one of the major City of Kathmandu. Hence, this research was conducted in the local poultry farm of Kirtipur. Histology work was done in the Pathology Research Laboratory of Central Department of Zoology in Tribhuvan University, Kirtipur followed by Teaching Hospital, Maharajganj (Figure 2)



**Fig 2:** Satellite map of Kirtipur (left) and Faculty of Science Block (right) of Tribhuvan University showing Central Department of Zoology.

## 2.2 Experimental Procedures

A total of 225 domestic fowl of different ages and breeds were collected either sacrificed or freshly dead birds from the local poultry farm during the period extended from March 2013 to April 2015. These samples were brought to Pathology Research Laboratory of Central Department of Zoology in Tribhuvan University, Kirtipur and subjected to clinical, postmortem and parasitological examinations (Figure 3).



**Fig 3:** A healthy domestic chicken *Gallus domesticus* moving around the farm

Birds were dissected open midventrally and different organs including alimentary canal were removed and kept in separate desired size petridishes where these organs were teased and cut open to search for parasites if any. Therefore, the gastrointestinal tract was subjected to routine examination to collect the gastrointestinal parasites, according to the procedure as described by Fowler [11]. Tapeworm were collected by the help of dropper and preserved in 10% formalin or cornoy's fluid for the identification. The other steps in this were fixation, staining, dehydration, de-alcoholisation and clearing, mounting and labeling. Ethical clearance and valid approval were obtained from the Government of Nepal, Ministry of Health before the commencement of the experiment.

## 2.3 Identification, Morphology and Histology

*Raillietina tetragona* were identified according to the keys and description given by Soulsby [12] and Yamaguti [13]. Morphology of *R. tetragona* was studied by preparing permanent slide according the methods as described by Cable [14]. For histological studies sections of the intestines were cut and fixed in 10% buffered saline which was then taken to the laboratory of Central Department of Zoology for histopathology. The intestinal segments were processed, dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned with a microtome at 5µm thickness, and placed on slides which were stained by the Harris haematoxylin and eosin method [15].

## 2.4 Examination Procedures

Light microscopy was conducted under Olympus Research microscope with lens combination of 7X, 10X, and 15X eye pieces and 4X, 10X, 20X, 40X and 100X objectives. The drawings for identification purposes were made to scale with the help of prism type camera Lucida. The Photographs were taken with the help of Sony Digital SLR Camera Model Number (DSLR-A200). Photomicrography was conducted with DP-12 Digital Camera attached to Olympus Research Microscope in the department of Zoology.

## 2.5 Statistical analysis

Data obtained from the prevalence study were analyzed using descriptive statistics and the results summarized as percentages. One way analysis of variance (ANOVA) was used to analyse the data generated from worm counts and variant mean separated by the Duncan's multiple range test. Probabilities (*P*) of 0.05 or less were considered as significant.

## 3. Results

### 3.1 Clinical Findings

The clinical examination of examined affected chickens showed symptoms varies in severity in accordance to age and breed. The clinical symptoms were ranged from subclinical to sever symptoms and death. The examined chickens showed dullness, emaciation, weakness, reduced growth and convulsions and some birds showed paralysis (Fig. 4). In addition examined layers and breeders showed uneven body weights and decreased egg production.



**Fig 4:** Stressed Chicken observed

The post mortem examination also revealed that all the carcasses had ruffled feathers and were moderately to severely emaciated. Seven out of 15 birds examined showed severe emaciation of the breast muscles with protrusion of the keel bone. The intestinal lumen (duodenum and jejunum) of all the carcasses when cut open were severely filled with whitish worms measuring about 15cm to 25cm in length as their anterior ends were firmly attached to the intestinal mucosal and dark red exudates (digested blood) was seen. The duodenal mucosa was thickened and hyperemic showing chronic catarrhal enteritis and hemorrhagic enteritis (Fig. 5).



**Fig 5:** *R. tetragona* recorded in the gastro-intestinal lumen during observations after dissection and post mortem.

### 3.2 Parasitological Findings of Cestode: *Railletina tetragona*

#### (a) Generic Diagnosis

Numerous proglottids were observed. Rostellum with hammer shaped hooks, suckers armed with minute deciduous or persistent hooks. Testes were numerous. Cirrus pouch was small. Genital pores unilateral or bilateral. Ovary was bilobed. Vitelline gland was compact. Egg capsule with one to several eggs were observed (Figure 6).

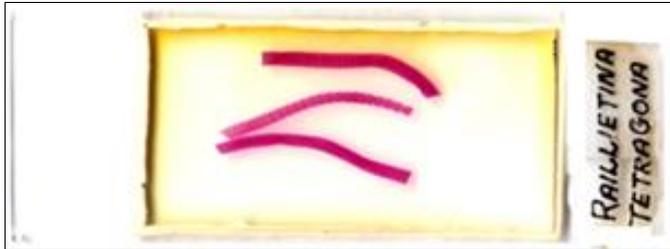


Fig 6: Slide of *R. tetragona*

#### (b) Species Diagnosis

Rostellar hooks in circular row. Genital pores unilateral and the egg capsules with several onchospheres. Testes were numerous and ovary was median.

### 3.3 Descriptions

*Railletina tetragona* found in small intestines of domestic fowls and in case of heavy infection its location to large intestine also. It is large, robust cestodes, measuring up to 35 cm long. The scolex is oval in shape and is smaller measures 0.17 mm in width. The rosetellum is armed with one or two rows of T- shaped hooks and suckers, which are oval and armed with 8-10 rows of hammer shaped hooks of different size. The scolex is followed by long very narrow neck. The mature segment is longer than broad and the common genital pores are single and being in front of the anterior 1/3 of the lateral margin of the mature segment (Fig 7a, b, c, d, e, f).

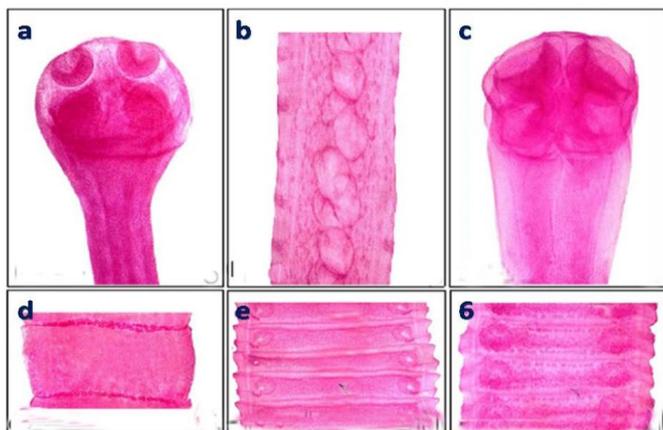


Fig 7: Different stages of helminthes parasite observed under the microscope

Table 1: Seasonal incidence of *R. tetragona* recorded during this investigation

Season	No of Chickens Examined	No of Infested Chickens	% of <i>R. tetragona</i> infestation
Spring	47	4	8.51
Summer	49	9	18.37
Autumn	56	11	19.64
Winter	73	7	9.59
Total	225	31	14.03

The Cestode *R. tetragona* also recorded during the different seasons (spring, summer, autumn and winter) and it was highest 19.64% parasites were recorded during autumn season while the infestations were as 18.37, 9.59 and 8.51 in the seasons of summer winter and spring respectively.

### 4. Discussion

Development of Nepal's poultry production could be divided into three distinct periods since its commencement in 1965. The period from 1965 to 1980 was primarily engaged in subsistence production, from 1981 to 2002 the period of commercialization and then onwards the period of competitiveness which is continuing to date. After six years of government efforts to commercialize poultry farming by establishing a central hatchery, a US funded project produced 1700 New Hampshire day old chicks in 1961, Ratna feed, Kantipur poultry farms and Joshi poultry farms imported hybrid chickens from India and distributed them to poultry farmers in 1964 [16].

In Nepal there are 9822 hatcheries located in 21 districts as compiled and reported in 2010/11 but there are no grandparent farms to date. Locations with high concentration of hatcheries are Chitwan (42), Kathmandu (16), Bhaktapur (8), Rupandehi (6) Kaski (3), Lalitpur (3), Kavre (3), Morang (3) and Dang (2). Similarly, the other twelve districts namely Sunsari, Kapilvastu, Rautahat, Sarlahi, Parbat, Banke, Nawalparasi, Parsa, Siraha, Dhading and Kailali contain one hatchery each. Among them, 72 hatcheries produce broiler day old chicks and the others layer day old chicks. The broiler and layer chicken market has grown by 18 and 17 percent, respectively in 2009/10 compared to the previous year and domestic demand is around 0.2 million to 0.25 million chickens per day. As derived from the compiled annual technical reports of Central Animal Quarantine Office, Budhanilkantha- Kathmandu the share of broilers among the parent stocks was 95.9% in 2005/06, 97.4% in 2006/07, 92% in 2007/08, 79.7% in 2008/09, and 96.96% in 2009/10, 93.12% in 2010/11 and 90.26% in 2011/12 [17].

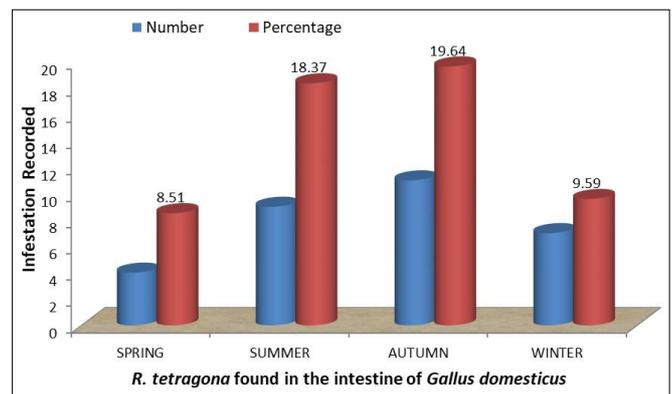


Fig 8: Domestic chicken *Gallus domesticus* infested with *R. tetragona* at poultry farm of Kirtipur village.

After extensive study of the bird host, *Gallus domesticus* for helminth parasitism in Kirtipur village during the present study, *R. tetragona* belong to Cestode of helminthes parasites were recovered (Fig. 8) with a moderately high prevalence (19.64%). These results when compared with those of other researchers around the globe indicate that this small, however isolated, valley does not figure out of the helminth infestation; but is an endemic area for helminth parasites in fowl. Yadav and Tandon [18] revealed 90.9% of helminth infections in subtropical high rainfall area of India. Mpoame and Agbede

[19] found 93.55% of domestic fowl infected with gastrointestinal helminthes. Eshetu *et al.* [20] found 91.01% chickens infected with gastrointestinal helminthes from Amhara region Ethiopia. Nokana *et al.* [20] during their survey of helminth parasites in backyard flocks in Michigan by litter examination also showed relatively high contamination rates. Oyeka [21] found 54.5% of chickens to be infected with helminth parasites in Anambra state in Nigeria. Ayesha reported a prevalence of 51.42% of *Raillitina tetragona* in domestic fowl from Doda district of Jammu and Kashmir. The present study reveals that single type infections were more prevalent than multiple type infections. Multiple type infections with helminthes in domestic fowl were also observed by researchers in the references [22]. In this study, majority of the host birds harboured single type of infection of helminthes which suggest that the prevailing environmental conditions and free range management systems are favourable to many species of helminth parasites [23].

## 5. Conclusion

The findings of this study show that helminthes infections are endemic in the study area, with *Raillitina tetragona* been the most prevalent among the indigenous domestic chicken *Gallus domesticus* detected. Two-third of the sampled farms was infected with *Raillitina tetragona* parasite. There was a low mean intensity of infections, and this will not rule out the economic effect, helminthosis cause on production. A number of factors were significantly associated with the positivity of helminthes infections. Histopathological studies revealed that the intestine of infected fowl showed haemorrhages, less growth of villi, ulcerations, incisions, nodular growth, eroded mucosal epithelium and load dependent mild to moderate enteritis. In general, the degree and extent of enteric changes corresponded to the parasitic load. Attachment of the parasite caused traumatic lesions which might have favoured secondary bacterial infection and hence more severe cellular reaction in the infected area was found. However delineation of local effects and species specific studies are needed. The most heavily infected segment of the intestine was the lower small intestine followed by the duodenum. The least populated segment was the rectum. This study will be essential for policy-making in other to improve poultry production in Nepal as poultry occupy a pivotal aspect of the national livestock sector.

## 6. Recommendations

Farmers raising fowl should improve feed provisions to their animals for good health conditions that confer some level of resistance against cestodiasis. Control of cestodiasis either by treatment interventions made by individual farmers or through community involvement is anticipated to increase farm level productivity and household income. Awareness programmes should be organised to educate the farmers for prevention and treatment methods. Lastly the current study recommends an integrated approach to combat cestodiasis which includes broader study of other risk factors like intermediate host habitat and effect of climate.

## 7. Acknowledgement

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