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Raillietina echinobothrida infection in black crows (*Corvus splendens*) at Hisar (Haryana)

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Abstract
The black crow is one of the common avian birds observed around the human habitat. The bird harbour series of endo-parasites because of its omnivorous eating behaviour. The present study reports the presence of *Raillietina echinobothrida* infection in the intestine of dead black crows at Hisar, Haryana. The study further elaborate the method of collection, fixation, preservation, staining, differentiation, dehydration, clearing and mounting of adult worm and detail morphological description of the *Raillietina echinobothrida* cestode.

Keywords: Black crows, Hisar, *Raillietina echinobothrida*

Introduction
Indian crows are commonly black in colour known for their intelligence, adaptability and for their loud, harsh "caw". The black crow belongs to order Passeriformes and is one of the most common birds of the crow family which is of Asian origin but now found in many parts of the world. Male and female house crow look alike, although males are slightly larger. They are slender glossy black birds with a charcoal grey collar and underparts. House crows have the reputation for being invasive species and, once established, are probably impossible to eradicate due to their behavioural and ecological flexibility combined with their legendary intelligence. These birds have good communication and problem solving skills (Tidemann and Gosler, 2010) [11]. The house crow is already recorded in the urban residential localities on houses, parks, electric wires, trees approaching residential localities in Hisar (Devi and Chopra, 2017) [5]. They are highly opportunistic birds with omnivorous diet which includes small insects like ants which act as intermediate host for *Raillietina* spp. and *Cotugnia digonopora*. Among the different *Raillietina* spp., *R. echinobothrida* is listed as one of the most pathogenic tapeworm occuring in small intestine of birds in most parts of the world (Bhatia *et al*., 2014) [3]. Under heavy infection, it causes conspicuous intestinal nodules with characteristic hyperplastic enteritis associated with the formation of granuloma. This parasite is responsible for 'nodular tapeworm disease' in poultry and other birds (Nadakal *et al.* 1973) [7]. Intestinal nodules often result in degeneration and necrosis of intestinal villi, accompanied by anaemia with a significant increase of total leukocyte counts and decrease of total serum protein. Studies on incidence of gastro-intestinal parasites in black crow in India are scanty. The present report highlights collection, fixation, preservation, staining, differentiation, dehydration, clearing and mounting of adult *Raillietina* worm from small intestine of a black crow examined at Department of Veterinary Parasitology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Science (LUVAS), Hisar, Haryana. Some worms were also preserved in 70% alcohol for further molecular characterisation.

Materials and Methods
Study area
Hisar district is located in Haryana with 29° 5'5"N latitude and 75° 45'55"E longitudes in western Haryana (Fig. 1). City is located 164 Km north-west of Delhi on national highway, NH-10. It has an average elevation of 215 m (705 ft) above mean sea level. Total area of Hisar is 3788 square kilo-meter, contain 9 blocks and 281 villages (Fig. 1). Annual average maximum and minimum temperature is 31.5 °C (88.7 °F) and 16.2 °C (61.2 °F), respectively. Relative humidity varies from 5 to 100%. The varied habitats support a rich avian fauna.

Fig 1: Studied area (Shows in red colour)

Eight dead black male crows were collected from road side below the electric wires during early morning hours in the month of December, 2017. These birds were brought to the department of veterinary parasitology, College of Veterinary Sciences, LUVAS, Hisar for post-mortem examination by veterinary students. The examination of intestine in one of these crows revealed many dorso-ventrally flattened, live, white coloured, ribbon like, long segmented tape worms with body divided into scolex, small neck and a long strobilla. These worms were embedded in the mucosa of jejunum part of small intestine. The worms were collected in warm normal saline solution in a large petridish for removal of debris, clearing and further examination in laboratory. The gross examination revealed adult parasites of around 15-20 cm length (Fig. 2). The adult parasites were processed as per the description of Soulsby (1982) [9]. These parasites were washed in luke warm water, fixed and preserved in 70% alcohol for two days. The preserved cestodes were separated into scolex, mature segments and gravid segments. The different parts of parasites were stained using borax carmine (alcoholic stain) (Hi-Media Laboratories Private Limited, Mumbai) and differentiated using 1% acid alcohol (prepared by adding 1 ml of concentrated sulphuric acid in 99 ml of 70% ethyl alcohol). Differentiation was done until the internal organs were visible. The parasites were dehydrated in ascending order of alcohol i.e 70%, 80%, 90% and absolute alcohol for 1 hour each, cleared in clove oil, dipped in xylene and further mounted in DPX on a glass slide. The parasite was covered with coverslip avoiding any air bubble. The slides were dried for 7-10 days at 27ºC in an incubator. Extra mounting material was removed after solidification. The morphological haracters were identified using the key of Urquhart *et al*. (1996) [12].

Fig 2: Adult *Raillietina echinobothrida* in petridish

Results and Discussion

The percentage of infection of *R. echinobothrida* in Black Crows (*Corvus splendens*) at Hisar is given in Table 1. Grossly, characteristic tapeworm structure, composed of a series of ribbon-like body segments, gradually enlarging from the anterior end towards the posterior. The gravid proglottids were frequently separated in the middle forming small windows which were visible at the posterior part of each gravid segment. Microscopically, after preparing a stained slides, the scolex showed suckers which were circular in outline and armed with many rows of small hooks. The rostellum was heavily armed with two rows of hooks (Fig. 3). The mature segment showed lobed ovary at the centre, kidney shaped vitelline glands at posterior end of ovary, single set of genital organ in each segment and unilateral or irregularly alternating genital pores (Fig. 4). The gravid segments had small windows in between two segments at the post part of worms (Fig. 5). The uterus in gravid segments contained egg capsule containing 8-10 eggs per egg capsule (Fig. 6). This morphological description confirmed it to be *R. echinobothrida*. Similar were the description of Radha *et al*. (2006) [8], who described *R. echinobothrida* that infects *Gallus domesticus* (fowl). Although there are many reports of intestinal parasites of crow by many authors (Bilqees and Khan, 2005; Andrews and Threlfall, 1975; Bano, 1970; Jones, 1968 and Suleman and Khan, 2016) [4, 1, 2, 6, 10] but as per the available literature this is a rare report of presence of *R. echinobothrida* in black crow.

Table 1: Infection of *R. Echinobothrida* in Black Crows (*Corvus splendens*) at Hisar

S. No.	Number examined	Number positive	Percentage
1.	8	1	12.5%

Fig 3: Scolex showing four suckers and round rostellum

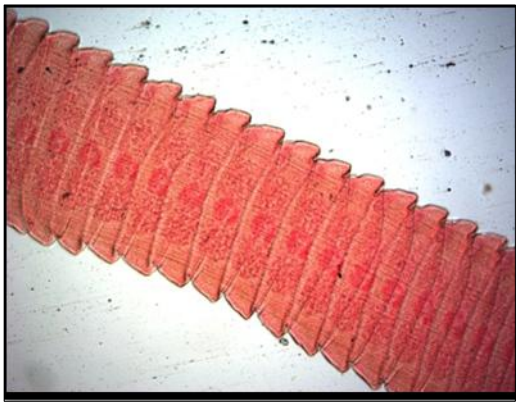

Fig 4: Mature segment showing genitalia

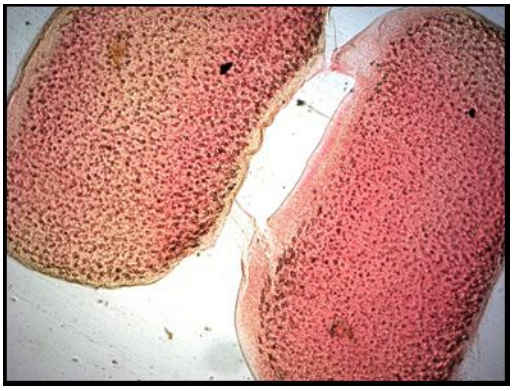

Fig 5: Small windows in between two gravid segments segments

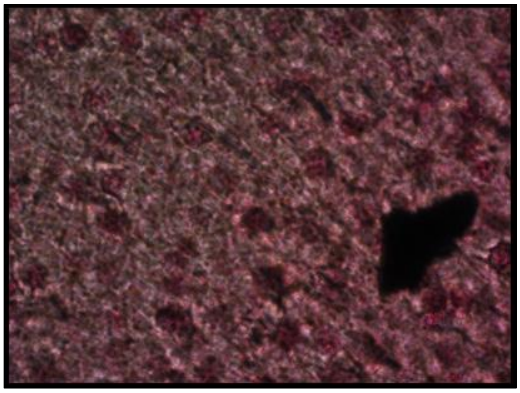

Fig 6: Egg capsule containing 8-10 eggs per egg capsule

Summary
Out of eight dead black male crow examined, one was found infected with tapeworm, which after processing was confirmed to be *R. echinobothrida*.

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