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In vitro analysis of, *Ascaridia galli (*nematode) Interaction with Gut Microbiota of Chicken

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Abstract

Helminth infestations, are a regular menace in cattle and poultry. Wild and in-house animals are frequently infected with different helminth species. In recent times it was proposed, that, the greater success of these helminth infections, may be attributed to greater diversity of intestinal microbiome, which seems to promote the survival of these parasites**.** In the present study, *in vitro* analysis of a helminth parasite, *ascaridia galli* with that of common, gut microbes was tested. For the, present study*, ascaridia galli* powder extract was used. My results, showed that *ascaridia* extract, showed no anti bacterial activity in *in vitro* experimentation, against the common, intestinal bacterial species, such as *Staphylococcus aureus, Streptococcus pneumonia, Proteus vulgaris Klebsiella pneumonia, Escherichia coli.* This study, suggests, that, *Ascarida galli*, the nematode parasite of chicken intestines seems to be tolerant towards the gut bacteria and seem to coexist in a mutulistic association.

Helminth infections, are quite common, in cattle and the poultry. Chicken are frequently affected by nematode infection of *Ascaridia galli*, which inhabits the small intestines of chick. This is the most common infection infecting the poultry all round world[10]. Life cycle of *Ascarida galli* lasts for 21 days[6] and the larvae migrate to the intestinal mucosa or remain in lumen. The parasite is known from times immemorial suggesting that these parasites are quite successful in evolution. The successful establishment of infection, laying of eggs by the gravid female, sustenance to larval forms and maturing in to adults may be attributed to suitable selection of host and to certain extant, may be due to gut bacterial species, which might provide, an amicable environment, for the sustenance of the parasite. In recent times, studies, related to the association of helminthes with that of gut microbiota, are gaining much importance. There are instances, where the helminth infections are known to alter the composition of gut bacteria and also their metabolic

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capabilities[7] helminth and gut microbial studies by authors, like[11], suggest that, soil transmittable helminthes (STH) are known have influence on the intestinal microbiome .Another study, by Hayes *et.al*[2], suggests, that, for successful infection, of *Trichuris muris*, in mice is dependent upon the existence of specific bacterial species of mice intestines. In addition, to these observations, in another study by Johnson & Reid[3] on *Ascaridia galli,* has shown that, this nematode, infection in chicken was found to be high, in the presence of gut bacterial biome, suggesting that, gut bacteria seem to promote the survival and sustenance of parasitic infections. Based upon these studies, it was proposed to investigate the *in vitro* interaction between *Ascaridia galli* and intestinal microbiota.

Infected chicken intestines, were procured from source, (slaughter house). They were thoroughly cleaned and the parasites, *Ascaridia galli* were identified and removed. They were placed in saline solution in a petridish and dried in a incubator for 3-4 hrs. Later the dried parasite, was grounded in to powder using motor and pestle. The powdered extract was collected in to a small vial and used for further experimentation.

Cleaning and sterilization :

The glass wares used in the present study were cleaned with cleaning solution and sterilized in hot air oven to 180 ^{O} C for 1hrs. All nutrient media were sterilized by autoclave at 121ºc, 15 lbs for 15-20 minutes.

Cultures of pathogens:

The bacterial strains investigated were Staphylococcus aureus [MTCC 96], Streptococcus pneumonia [MTCC 655], Proteus vulgaris [MTCC 1771], Klebsiella pneumonia [MTCC 109], Escherichia coli [MTCC 9537of the cultures were purchased from the institute of microbial technology, Chandigarh, India . All these bacterial were maintained on freshly prepared Nutrient agar slant for bacteria at 4°C. Mueller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer ' s instructions. Immediately after autoclaving, allow it to cool in a 45 to 50 ^{o}C water bath and Pour the freshly. Prepared and cooled medium into glass or plastic, flat-bottomed petridish on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 25 to 30 ml for plates with a diameter of 100 mm. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8^{o}C) and a representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35 ^{o}C for 24 hours or longer.

Preparation of inocula : To standardize the inoculum density for a susceptibility test, a BaSO 4 turbidity standard, equivalent to 0.5 McFarland standard or its optical equivalent (*e.g.*, latex particle suspension), should be used. Then the inocula of bacteria were prepared from the 12 hrs broth cultures and standardized to10 8 Cfu/ml. The infective dose for most organism are 10 5 Cfu/ml. Moisture, If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35^{o}C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist,

but no droplets of moisture should be apparent on the surface of the medium or on the petridish covers when the plates are inoculated.

Agar well diffusion method : The antibacterial studies of the Animal extract were carried out by Agar well diffusion method The animal extract were dissolved in sterile distilled water to get concentration of 100mg/ml solution. Ciproflaxacin 0.5mg/ml were used as a standard drug for this test. The antibacterial activity was evaluated by employing 12-18 hrs cultures, of *Staphylococcus aureus* [96], *Streptococcus pneumoniae* [655], *Proteus vulgaris* [1771], *Klebsiella pneumoniae* [109], *Escherichia coli* [9537] using Muller - Hinton agar medium . Wells [6mm] are made into the agar plate with sterile well borer. 25µl, 50µl, 75µl, 100µl of the test sample and 25µl of the standard solution were transferred to the wells in the microorganism inoculated plates aseptically and labeled accordingly. Sterile distilled water act as a negative control. Then the plates were maintained at room temperature for 2 hrs enabling the diffusion of the solutions into the medium. The petriplates were incubated 37ºC for 24 hrs for antibacterial screening. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm). At least three replicates were carried out for the extract against each of the test with the organisms.

In the present experimentation, results, showed that the extract prepared using *Ascardia galli* was found to be ineffective against the gut microbiota. Bacterial species namely *Staphylococcus aureus, Streptococcus pneumoniae, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli,* were tested, against the parasite extract using agar diffusion method and standard ciprofloxin was used as the standard. The results are presented in the table-1. and standard ciprofloxin showed the antibacterial activity, measured as minimum inhibitory concentration (MIC) against different bacterial species as shown in fig-1. As mentioned, *Ascaridia galli* powdered extract of different concentrations, like 25µl , 50µl 75µl and 100µl when tested against the bacteria, it showed no antibacterial activity, suggesting that it might coexist in the host intestine along with the gut microbiota.

Table-1

S.No	MTCC Code	25µl	50 µl	75 µl	100 µl	Std Ciprofloxin
1	*Staphylococcus aureus (96)*	-	-	-	-	34
2	*Streptococcus pneumoniae (255)*	-	-	-	-	38
3	*Proteus vulgaris (1771)*	-	-	-	-	28
4	*Klebsiella pneumoniae (109)*	-	-	-	-	28
5	*Escherichia coli (9537)*	-	-	-	-	24

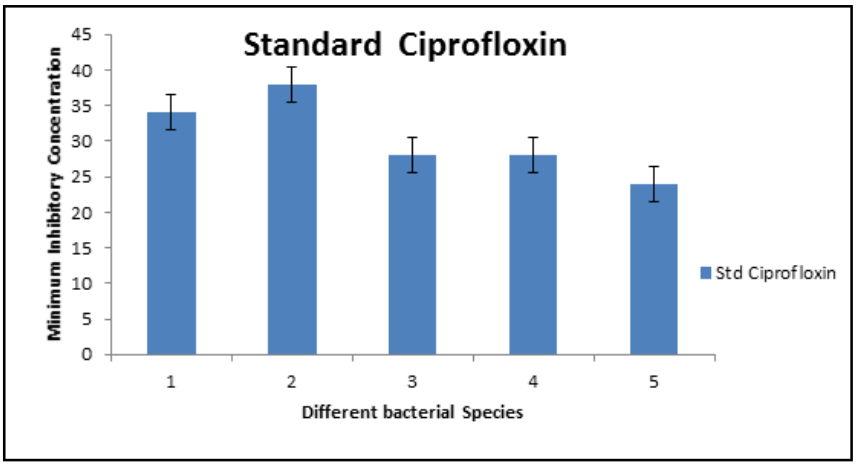

Fig. 1

In the present, study, *in vitro* analysis, of antibacterial activity of the powdered extract of nematode parasite *Ascaridia galli* was studied. Gut bacteria, belonging to species *Staphylococcus aureus, Streptococcus pneumoniae, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli*, were tested using agar diffusion method. The results, showed that there was no anti bacterial activity presented by the parasite extract. These results suggest that, gut microbiota and the helminth parasite seem to engage in cooperative existence, in the intestines of chicken. This observation, coincides, with that of the Reynold *et. al,*[8] who have suggested that there seems to be a co evolutionary relationship between the bacterial species and intestinal helminthes which cooperate to create amicable environment for both the species (Helminthes and bacteria) in the host intestine. Certain studies by Lee *et.al*,[4] and Cantacessi *et al.*[1], suggest that there was measurable increase in the microbial diversity and also their number when host animals were infected with helminth parasites. Studies by Zaiss &. Harris,[11] on soil transmitted helminthes, proposed that, intestinal helminth parasites are known to constantly synthesize certain secretions, which are known to promote the growth of gut microbial species. It is interesting to note that, in another study by Midha *et.al*,[5] and Thivierge *et.al.,*[9] proposed, that the helminth parasites, such as *Ascaridia suum*, and *Fasciola hepatica*, are known to produce antimicrobial peptides, which seem to influence the intestinal bacterial population. From these studies, it may said be that, different helminth species interact differently towards gut bacteria. Some worms, tend to promote the growth of intestinal microbes, and certain parasites, might be antimicrobial, and neutralize their populations. In the present study, it was observed that there was no antimicrobial activity noted against the bacterial species, suggesting that *Ascaridia galli*, a nematode of chicken intestines, seems to tolerate and coexist along with the gut microbial species and aid in survival of the host animal.

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