



**SEM STUDY OF THE EFFECT OF ANTIPARASITIC DRUG
IVERMECTIN ON THE *ASCARIDIA GALLI***

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ABSTRACT

Poultry has a crucial place in India, as the eggs and chicken meat are rich sources of high quality proteins, vitamins and minerals to balance the human diet. *Ascaridia galli* infection occurs worldwide in all poultry of all ages. In severe infections, intestinal blockage can occur. At high levels of infection, there is loss of blood, reduced blood sugar content, retarded growth and greatly increased mortality. In the present paper, the *in vitro* activity of antiparasitic drug ivermectin was investigated on the cuticle of *A. galli* under SEM .It was observed that cortical layer was severely affected by the action of anthelmintic ivermectin. The present study revealed shrinkage and rupturing of cuticle. Annuli showed disorganization. The lips showed compact and fenestrated appearance when treated with ivermectin under SEM.

KEY WORDS: *Ascaridia galli*, ivermectin, *in vitro*, SEM, anthelmintic.

In the last two or three decades, poultry has made tremendous strides and now India is one among the top five chicken producing countries with the annual production of 530 million broilers and among the top six egg producing countries in the world with the annual production of 33 billion eggs. The National Committee on Human Nutrition in India has recommended per capita of 180 eggs and 10.8 Kg. meats. It is estimated that by year 2012, the requirement will be 180 billion eggs and 9.1 billion Kg. poultry meat while the estimated production may only be around 46.2 billion eggs and 3.04 billion Kg. poultry meat.

India is the native place of the wild jungle fowl. *Ascaridia galli*, one of the most common parasites of chickens, causes heavy economic losses in them by reducing their growth rate and meat production. The total loss in India due to *A. galli* infection in growing chicks and egg layers have been estimated about 150 million and 3000 million respectively. The disease caused by *A. galli* is “ascaridiasis” affecting the general health of the birds. These parasites occur commonly in duodenum and small intestine of fowl, turkey, guinea fowl, goose and various wild birds, many times causing blockage of gastro-intestinal tract. Presence of *Ascaridia galli* leads to various or highest degree of pathogenicity. When these worms are present in large number, they may kill the host. These infected chickens reveal retarded growth and poor utilization of feed even if balanced diet is given. Besides all these, presence of nematodes also causes malnutrition in hosts, which is one of the major causes of decreased return of products derived from animals. (WHO, 1967; TRIPATHY *et.al*, 1971; GUPTA *et al.*, 1977; NASHEIM AND FORSUM, 1980).

Besides pathological, nematode cuticle is also extremely important from immunological and immunopathological point of view. Therefore, it is necessary to know the clear structure and modifications

of the outermost layer that is cortical region, which remains in contact with the intestine of the host. The resolution of the cortical region would help in understanding various surface antigens. As above roundworms are intractable parasites and feed on mucous membrane and hence the anthelmintic also affect the cuticle and such effects need further researches.

Although a good deal of information is now available about anthelmintics and their mode of action but the parasites are still flourishing. There is a great need, therefore, to search new drugs. The designing, formulation and marketing of new anthelmintics require studies regarding various aspects of the action of the anthelmintic not only on the parasite but also on the host.

MATERIALS AND METHODS:

The parasites used in the study were collected from intestine of fowl slaughtered freshly in local abattoir. After removing, the parasites were washed with saline water thoroughly. To study the effect of ivermectin, two groups of parasites A and B were made.

In group A, ten parasites of mixed distribution were kept in petridish containing lock-lewis solution for control. In group B, ten parasites *A.galli* of mixed distribution were kept in petridish containing lock-lewis solution with ivermectin in 50 µg / ml concentration. These petridishes were kept at 37°C for incubation. After incubation for 8 hours, the parasites were kept into the modified Karnovskys fluid, used as a fixative. The parasites in fixative were kept at 4°C for 5 to 6 hrs. After fixation parasites were transferred into 0.1M cacodylate buffer solution and again washed with sodium phosphate buffer solution then fixed parasites were dehydrated. Subsequent dehydration was carried out through ascending concentration of acetone up to pure acetone. Following the standardized scanning electron microscopic protocols developed by Dey *et al* .26 and Roy and Tandon, 27 specific for helminth parasites, the specimens were processed. The dried materials were placed on metal stubs and sputter-coated with gold in a fine-coat ion sputter, JFC-1100(JEOL). The gold-coated specimens were observed under scanning

electron microscope (LEO 435 VP) at an electron accelerating voltage of 20 kV. Simultaneously photographs were taken.

OBSERVATIONS :

1. SCANNING ELECTRON MICROSCOPIC (SEM) STUDIES OF *A. GALLI*

CONTROL WORMS: Under SEM *A. galli* worms were studied. The mouth was surrounded by three prominent denticulate lips having smooth cuticle preferably for the anchorage of parasites on the intestinal mucosa. The lips were of two types, one mid-dorsal which was broadly elliptical and two latero-ventral and oval in shape. Each lip was wide at the base with gentle tapering toward the apex. SEM study revealed that each lip consisted of three distinct lobes; one median and two lateral lobes at the side. Three lobes fuse together form a cup like structure (Fig4) enclosing central circular mouth, SEM study of cuticle revealed that it was smooth. A series of continuous transverse annulations with distinct striations were observed originating from the cephalic region to the posterior end of the body.

SEM studies revealed that striations were fine transverse grooves in the form of parallel thick concentric oblique rings running completely around the body. Under SEM it

was observed that concentric rings converged or interrupted at points at the longitudinal ridges. Annulations were observed as deep transverse groove occurring as regular intervals giving the body a segmented appearance. Annulations were finally divided into small sub annuli (Fig. 5). In the SEM study, the cephalic region of male worm revealed the same structure like females (Fig.1,2,3,4,5,6).

SEM STUDIES OF *IN VITRO* IVERMECTIN TREATED WORM *A. GALLI*

(At 50 µg/ ml concentration)

Under SEM ivermectin incubated worms revealed shranked and disturbed armature of the cephalic region. The SEM study of cephalic region showed the shrinkage and disorganization of lips. The cuticle was ruptured. Major changes were observed in the external cortical layer. The inner cortical layer was intact. Transverse striations were separated. Lips showed fenestrated appearance. Rupture of cortical layer was very distinct. Normal pattern of striations was also disturbed. The dorsal and two sub-ventral lips compact and fenestrated (Fig.7).Below cephalic region shrinkage of cuticle was observed. Cuticle became wrinkled and transverse striations were more prominent due to shrinkage (Fig. 8).

In the magnified dorsal side of posterior region cuticular striations showed disorganization of annuli and disturbed transverse striations. The cuticle was ruptured at several places. Ivermectin did not induce any crack and rupture of cuticle in this part (Fig. 9).Under high magnification of mid-dorsal region, annuli and subannuli were distinctly observed and cuticle showed ruptured external cortical layer and disturbed transverse striations. In another view of posterior region wide and narrow band like separation of transverse striations was observed (Fig. 10).

Vulva was not observed in the study due to position of the parasite on the stubs. The external cortical layer was observed to be ruptured (Fig. 11).

At certain places external cortical layer was observed to be fenestrated and ruptured but internal cortical layer was completely intact (Fig. 12).Tail region of the female worm was not affected by ivermectin (Fig. 11,12).

DISCUSSION: The nematodes are generally intestinal parasites and cuticle always remains in touch of the host and it is metabolically active and morphologically specialized for selective absorption of nutrients and osmoregulation .Then passive diffusion of anthelmintics would probably be responsible for destructive change and deformation of the nematode body surface. The cuticle of a large number of nematodes was studied by CHITWOOD (1937) and BIRD (1957). The present SEM studies have shown

that cortical layers were more severely affected by the action of anthelmintic ivermectin in the above worms. It revealed shrinkage, ruptured cuticle. Annuli also showed disorganization. The lips showed compact and fenestrated appearance when treated with ivermectin under SEM. These studies also support the earlier studies of BOGOLEPOVA AND SEMENKOV, 1973; DEREVYAGINA, 1978; ABDULAZIZOV, 1975.

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