

## IN VITRO EFFECT OF AN ANTHELMINTIC IVERMECTIN ON ASCARIDIA GALLI - A PARASITIC NEMATODE OF FOWL

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**ABSTRACT :** *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 an anthelmintic ivermectin was studied. The drug ivermectin induced evaginations, invaginations, shrinkage and disruption of the cuticle of *A. galli*. During the present study, severe alterations were also observed in uterus, ovary and testes induced by ivermectin. Eggs showed karyolysis, Karyokinesis and Karyorrhexis. Necrosis and edema were also observed in ovary.

**Key words :** *Ascaridia galli*, ivermectin, *in vitro*, anthelmintic.

### INTRODUCTION

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 2016, 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. *A. 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 gastrointestinal 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 for 8 hours.

To study the effect of anthelmintics on the body wall, intestine and other organs of parasites, the parasites were fixed in aqueous bouin's fluid for 24 hrs. The fixed worms washed thoroughly to remove excessive fixative. After washing the worms dehydrated in gradual steps of alcohol and then cleared in xylene. After dehydration, the worms were embedded in paraffin wax for the preparation of blocks and for section cutting (4µ to 5µ). After this sections were cut and stained in haematoxylin and eosin. These stained slides were studied under light microscope. Simultaneously the microphotographs were taken especially of body wall layers and intestine.

### OBSERVATIONS

#### Light microscopic studies on the body wall, intestine and reproductive organs of *Ascaridia galli*

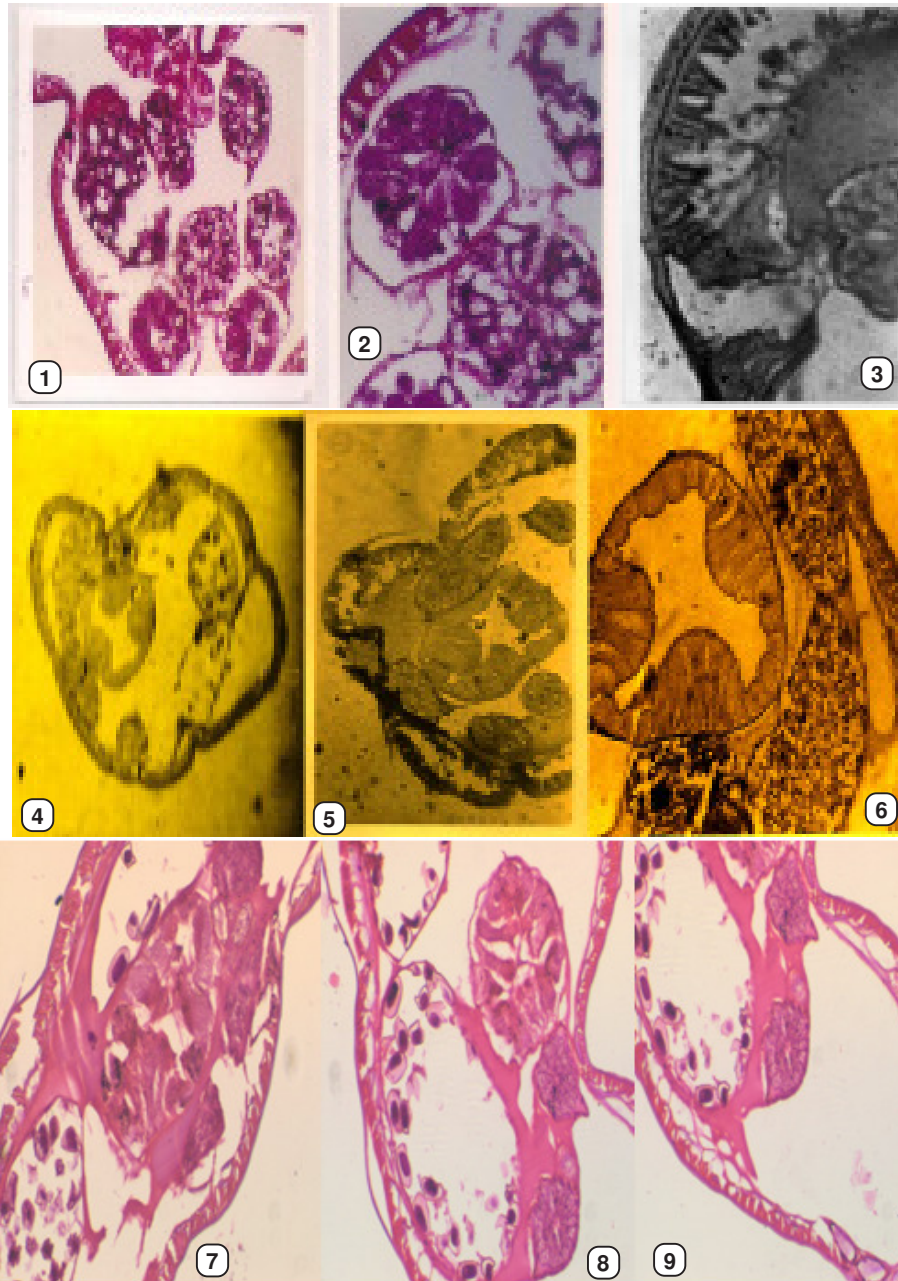
##### Histological structure of the control worms : (Figs. 1, 2, 3, 4, 5)

T.S. of *Ascaridia galli* showed cuticle, hypodermis, muscle layers, intestine and reproductive organs. The body wall of *A. galli* was composed of three layers: cuticle, hypodermis and muscle layers. The cuticle was made up of six layers namely external layer or cortical layer or upper layer deeply stained with 1.7 µ in thickness. The cortical layer was further made up of external cortical and internal cortical layers. Middle layer with no distinct constitutions, and then fiber layer, consisted of several layers of dense tissue settled on basement membrane. The hypodermis was present between cuticle and muscle layer. The hypodermis was of variable breadth. The hypodermis was thin and not well developed and of syncytial type. The hypodermis was observed as four bulging in the pseudocoelom in the form of four longitudinal chords; one dorsal, one ventral and two laterals. The lateral chords were well developed and larger than ventral and dorsal chord. The dorsal chord was comparatively larger than ventral chord. Each lateral chord was made up of a median glandular cell and nerve ganglion cell. The hypodermis was thicker as proceed posteriorly in the inter-caudal region as well as thicker in the four longitudinal chords protruded in the pseudocoel. Just beneath the hypodermis, muscle layer was present, which was made up of muscular part towards epidermis and protoplasmic part towards pseudocoel. The arrangement of muscle cells was holomyarian. Moreover, lateral cells

in the middle of the body were larger and more prominent becoming pyriform clusters or cup-shaped forming lateral hypodermal glands. Each muscle cell consisted of sub-hypodermal, contractile part and sarcoplasmic non-contractile part. In *A. galli*, the intestine was composed of endodermal columnar epithelial cells having microvilli called brushborder. It was observed that intestinal epithelial cells were enormously large at three places. The brush-border was of uniform thickness. The microvilli were compact finger like projections of the plasma membrane into the intestinal lumen. T.S. passing through the testes revealed that it was composed of outer basement membrane, sperm mother cells, primary spermatocytes, sperms and other supporting cells. T.S. passing through the ovary showed outer layer was made up of basement membrane, oogonial mother cells, oogonia and ova. The intestine was surrounded by coiled parts of two ovaries, two uteri and two oviducts. Numerous egg cells were present inside each of the uterus. The wall of uterus was comparatively thicker than ovaries and oviducts. Two uteri were filled with large number of ova.

##### *In vitro* ivermectin treated worm (*A. galli*) : (at 50 µg/ ml concentration)

T.S. passing through the worm after 8 hours of exposure with ivermectin, the cuticle revealed various histological changes. The external cortical layer and internal cortical layer showed disorganization. It was ruptured at several places due to shrinkage and penetrated into the pseudocoel at several places (Fig. 7, 13, 15 ). The external cortical layer showed wavy appearance due to invaginations and evaginations (Fig. 6, 7, 9,10, 15). At some places external cortical layer and internal cortical layer showed separation from hypodermis due to shrinkage. (Fig. 7, 15 ). On the other side cortical layer, fibrillar layer and hypodermis revealed fusion at various places. (Fig. 9, 10 ). Fiber, matrix and fibril layers of external cortical layer did not reveal distinct alterations except necrosis. The hypodermis revealed disorganization. It was ruptured and separated from cortical layer at several places (Fig. 13, 17) due to shrinkage in cuticle (Fig. 7, 15 ). It was observed that at some places hypodermis was homogeneous thin layer fused with internal cortical layer and somatic muscles (Fig. 9, 10, 12 14 ). The somatic muscles were severely affected. The typical platymyarian somatic muscles with meromyarian arrangement was disturbed and disorganized. The muscle cells appeared contracted at some places. (Fig. 7,15 ). The non-contractile part of muscle cells revealed distinct cloudy swelling. (Fig7, 8, 9, 10, 12 ,15 ) but contractile part of muscle cells was observed to be reduced in size (Fig. 7, 12 ,15 ). The



**Fig. 1 :** Microphotograph of T.S. passing through the male worm (Control) showing cuticle, hypodermis and testes lobes. 100 X. (H & E).

**Fig. 2 :** Microphotograph of T.S. passing through the male worm (Control) showing cuticle, hypodermis, muscle layer pseudocoelom and various cut parts of testes. Anterior of the testes filled with cut parts of the sperms. 200 X. (H & E).

**Fig. 3 :** Microphotograph of T.S. passing through the male worm (Control) showing cuticle, hypodermis and somatic muscle. 400 X. (H & E).

**Fig. 4 :** Microphotograph of T.S. passing through the male worm (Control) showing cuticle, hypodermis and somatic muscle, intestine and cut parts of ovary. 100 X. (H & E).

**Fig. 5 :** Microphotograph of T.S. passing through the female worm (control) showing cuticle hypodermis, somatic muscles, intestine and ovary. 100X (H&E).

**After 8 hours exposure at 50 µg/ml concentration**

**Fig. 6 :** Microphotograph of T.S. passing through the male worm (Ivermectin treated) showing changes in cuticle, hypodermis, somatic muscles, intestine and testes 400X (H&E).

**Fig. 7 :** Microphotograph of T.S. passing through the male worm (Ivermectin treated) showing broken cuticle, the number of eggs in uterus was less, eggs revealed necrosis, Karyolysis and Karyorrhexis and Karyokinesis, cloudy swelling in contractile part of muscle cells. 200 X. (H & E).

**Fig. 8 :** Microphotograph of T.S. passing through the female worm (Ivermectin treated) cloudy swelling, edema of ovary and uterus, nucleus of ova showed necrosis, Karyolysis, Karyokinesis and karyorrhexis. 200 X (H & E).

**Fig. 9 :** Microphotograph of T.S. passing through the female worm (Ivermectin treated) showing ruptured body wall, cloudy swelling of somatic muscles, necrosis, Karyolysis and Karyorrhexis of nuclei of ovum. 200 X (H & E).

contractile part also showed fusion but non-contractile part from band-like structure at certain places. (Fig. 6). Muscles cells showed edema (Fig. 8). Intestine also revealed altered structure. The endodermal columnar epithelial cells forming the brushborder revealed wide variations in their size at three places. There was no breakage of brush border and without vacuolization (Fig. 6). The uterus contained few eggs and their nuclei revealed karyolysis, karyokinesis and karyorrhexis (Fig. 7, 8, 15). Ovary and uterus showed edema. Due to dissolution of basement membrane uterus revealed cloudy swelling. The basal membrane and wall of oviduct were observed to be ruptured (Fig. 8). At few places ova were scattered outside the uterus due to rupture of basement membrane (Fig. 12, 17).

### DISCUSSION

In the present study, it was found that the anthelmintics induced changes in absorptive surfaces, cuticle, hypodermis, somatic muscles, intestine and reproductive organ. The exact mechanism by which the antiparasitic agents act in artificial culture is still not very clear (Pappas and Read, 1975; Sood and Kaur, 1982). But before knowing mode of action, it is essential to know the alteration caused by the different anthelmintics on the parasites.

The nematodes are generally intestinal parasites and cuticle always remains in touch of the host. The cuticle of a large number of nematodes was studied by Chitwood (1937) and Bird (1957). Under light microscope, the study of the T.S. of the body wall revealed that it was made up of six layers; external cortical layer, fiber layer, matrix layer and fibrillar layer. The cortical layer was further made up of external cortical layer and internal cortical layer. Middle layer or median layer settled on basement layer. Hypodermis and somatic musculature were distinctly observed. T.S. through intestine of the worms showed that it was composed of outermost basal lamella, endodermal columnar epithelial cells, terminal web and microvilli or brush border. In the intestine of *O. columbianum* a large number of crystals of variable sizes were observed in the intestinal cells. Intestinal crystals are inclusions in the intestinal cells of nematodes were also observed by Waller *et al* (1979), Bird *et al* (1978). T.S. of ovary showed that outer layer was made up basement membrane, oogonial mother cells, oögonia and ova. The uteri were observed to be filled with large number of ova. T.S. through the testes showed that it was composed of basement membrane, germinal epithelial layer, primary spermatocytes, secondary spermatocytes, spermatids, sperms and the supporting cells.

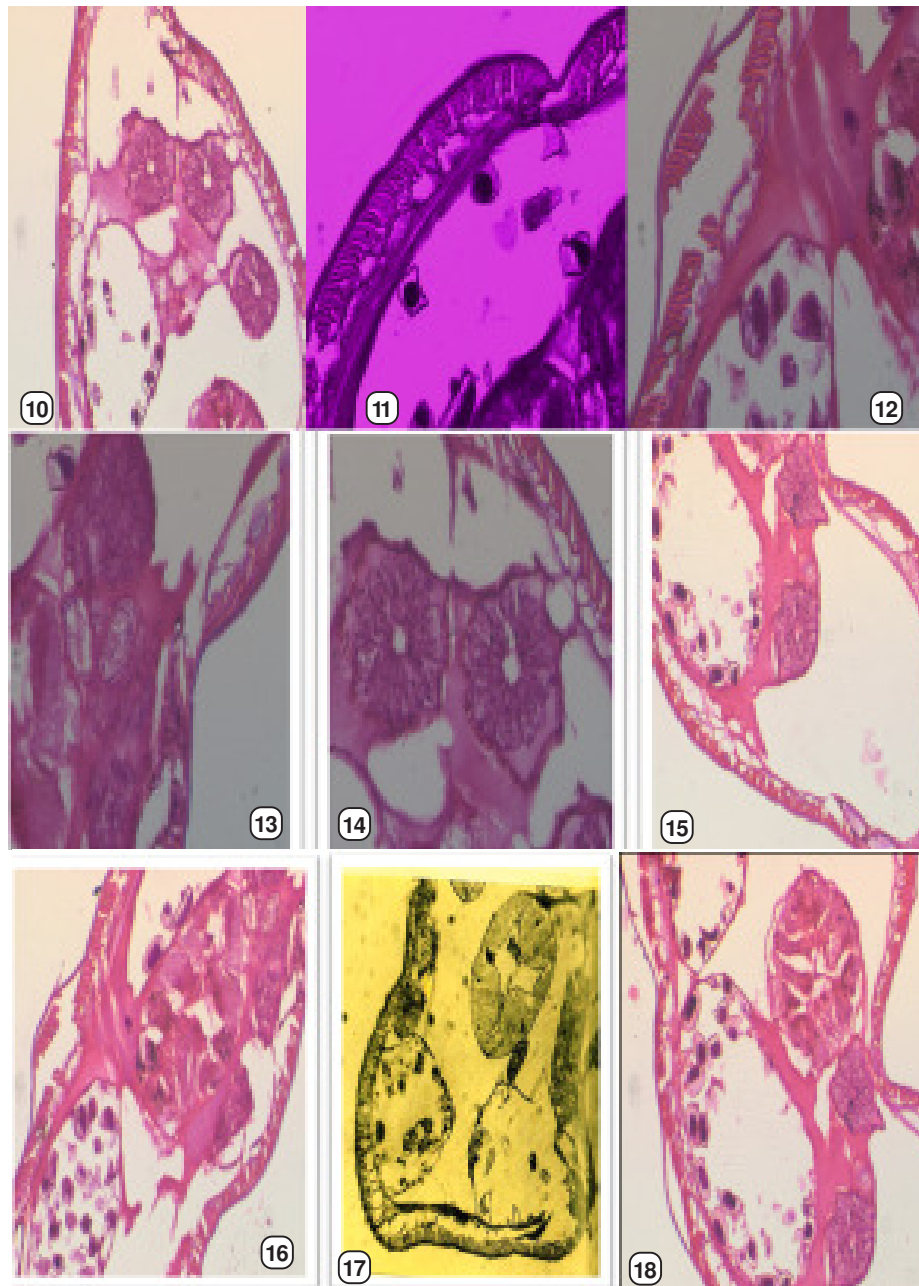
The result of the present study showed that the body wall and intestine were more severely affected by the anthelmintics. Similarly, the micromorphological changes in the body wall and intestine of *A. lumbricoides* by the action of piperazine were reported by Abulazizov (1975).

In the present study, ivermectin induced evaginations and invaginations, shrinkage and disruption of the cuticle of *A. galli*. At certain places separation of external cortical layer from internal cortical layer was observed. Similar degenerative changes were observed by Sood and Kaur (1982) in *H. contortus*. The present study also revealed that hypodermis was altered. At various places vacuolization of hypodermis was also observed and showed cloudy swelling, contraction and edema induced by ivermectin.

Similarly, various alterations were observed by Sood and Kaur (1982) in *H. contortus*. The morphological changes were also observed in cuticle, hypodermis and muscle layer of *A. galli* incubated at 37°C for 3, 6, 12 hours in 1:1000 solution of anthelmintic (Rachkaskaya, 1976). According to Sood and Kaur (1982), the intestine of the nematodes is an important site, through which the absorption of drug could occur. In the present studies, the intestine of the *A. galli*, showed various destructive and degenerative alteration caused by ivermectin

Pimenov and Shaidmuller (1971) have shown that the wall of epithelial cells was damaged in *O. dentatum* by phenothiazine but no damage was observed in *H. contortus*, *O. osterlegia* and *O. circumcincta*. Bagolepova and Semenov (1973) observed the destructive effect of carbon-tetra chloride and thiabendazole on the intestine of *S. trachea*. The irregular shaped endodermal columnar epithelial cells of intestine were degenerated and vacuolized with displaced nuclei in treated *A. lumbricoides* by El. Boulaqui *et al* (1979). Nollin *et al* (1974) also observed early degenerative changes such as vacuolization, disorganization and Karyorrhexis in muscle layer in encysted phase of *T. spiralis* by mebendazole. Pimenov *et al* (1975) observed changes in the alimentary canal of *Dictyocaulus filaria* induced by Cyacetazide iodine and laxurane diethylcarbamazine. Early degenerative changes in the intestine of *A. suum* after treatment with mebendazole comprise vacuolization, decrease number of microvilli, swelling clustering and disruption (Borgers and Nollin, 1975). Ruptured microvilli, vacuolization of intestine and collapsed lumen were observed by Zintz and Frnak (1982) in *H. spumosa*.

During the present study, severe alterations were also revealed in uterus, ovary and testes induced by ivermectin.



- Fig. 10 :** Microphotograph of T.S. passing through the female worm (treated) showing disorganized cortical layer, hypodermis and somatic muscles, edema of muscle cells and decreased number of ova in uterus. 200 X (H & E).
- Fig. 11 :** Microphotograph of T.S. passing through the female worm (Ivermectin treated) showing changes in body wall, scattered ova in pseudocoel, necrosis of nucleus. 200 X (H & E).
- Fig. 12 :** Microphotograph of T.S. passing through the female worm (Ivermectin treated) showing degeneration of body wall and cloudy swelling of somatic muscles. 100 X (H & E).
- Fig. 13 :** Microphotograph of T.S. passing through the female worm (Ivermectin treated) showing severely damaged body wall, cloudy swelling in somatic muscles, necrosis karyolysis karyokinesis and karyorrhexis of nuclei of ova. 200 X (H & E).
- Fig. 14 :** Microphotograph of T.S. passing through the female worm (Ivermectin treated) showing damaged and disorganized body wall, cut part of ovaries and eggs. 100 X (H & E).
- Fig. 15 :** Microphotograph of T.S. passing through the female worm (treated) showing ruptured body wall, cloudy swelling of somatic muscles, necrosis, Karyolysis and Karyorrhexis of nuclei of ovum. 200 X (H & E).
- Fig. 16 :** Microphotograph of T.S. passing through the female worm (treated) showing disorganised cuticle, ruptured hypodermis, cloudy swelling in somatic muscles and uterus. 200 X (H & E).
- Fig. 17 :** Microphotograph of T.S. passing through the female worm (Ivermectin treated) showing evaginated cuticle, blunting of contractile part of somatic muscles, haemorrhage in intestine. 280 X (H & E).
- Fig. 18 :** Microphotograph of T.S. passing through the female worm (treated) showing separation of different layers of cuticle, cloudy swelling, edema of ovary and uterus, nucleus of ova showed necrosis, Karyolysis, Karyokinesis and karyorrhexis. 200 X (H & E).

Eggs showed karyolysis, Karyokinesis and Karyorrhexis. Necrosis and edema were also observed in ovary. Similar changes were reported by Bogoyalenskii *et al* (1975 b, Derevyagina *et al* (1975 b)

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