Volume 66, Issue 5, 2022
Journal of Scientific Research
of
The Banaras Hindu University

Secretory acetylcholinesterase activities in fresh *Ascaridia galli* adult (Nematoda), excretory/secretory products, post-secretory parasite and gastrointestinal tissue of host *Gallus gallus domesticus*: A preliminary comparative study in host-parasite relationship.

Sulagna Maity[1], Subhoshree Mondal[2], Arup Kumar Ghosh[3] and Ipsit Chakrabarti[*4]

[1]Parasitology and Immunology Unit, Postgraduate Department of Zoology, Dinabandhu Andrews College (affiliated to University of Calcutta), Kolkata-700084, India. Email: sulagna.maity@gmail.com.ORCID: 0000-0003-0314-2429
[2]Parasitology and Immunology Unit, Postgraduate Department of Zoology, Dinabandhu Andrews College (affiliated to University of Calcutta), Kolkata-700084, India. Email: pritharockz@gmail.com
[3]Parasitology and Immunology Unit, Postgraduate Department of Zoology, Dinabandhu Andrews College (affiliated to University of Calcutta), Kolkata-700084, India. Email: Arupkg77@gmail.com
[*4]Parasitology and Immunology Unit, Postgraduate Department of Zoology, Dinabandhu Andrews College (affiliated to University of Calcutta), Kolkata-700084, India. Email: ipsitchakrabarti2011@gmail.com

Abstract:**Acetylcholinesterase (AChE), an esterase enzyme, terminates synaptic transmission by hydrolyzing the neurotransmitter acetylcholine into acetate and choline. Found in membrane-bound form, AChE has also been reported to be secreted in soluble form by numerous enteric helminth parasites. Secretory AChE is considered to be an immunomodulator that may promote the survival of parasites against the host's immune response. Our aim was to investigate the immunomodulation and survival strategy of helminth parasites *Ascaridia galli* inside the host *Gallus gallus domesticus* through analysis of AChE activity. The parasites, host intestinal tissue and post-secretory parasites were subjected to centrifugation, followed by AChE activity determination. The AChE activities among intestinal tissue, fresh parasites, excretory/secretory(E/S) product and post-secretory parasites were even compared. An inverse relationship between the reduced enzyme activity of the host's intestinal tissue and the increased enzyme activity in the parasite was observed. Our study indicates that the increased AChE activities in fresh parasites, post-secretory parasites and E/S product are their survival strategy as well as a part of their adaptive features vis-à-vis the hostile environment of the host'sintestine.**

Index Terms:**Acetylcholinesterase, *Ascaridia galli*, E/S product, *Gallus gallus domesticus*, Helminth, Immunomodulation**

[*]Corresponding Author

I. Introduction

Mostof the helminth parasites are adapted to the microenvironment of the hosts' gastrointestinal tract (CDC, 2018). In this environment, a certain life cycle stage of parasites synthesizes and secretes specific molecules to combat the defense mechanisms like activities of the digestive enzymes, peristalsis as well as to invade the immune system of the host (Hayunga, 1991). Helminth parasites are capable of manipulating host's immune system by releasing various molecules into their host organism and these molecules are referred to as excretory-secretory product or E/S product (Harnett, 2014).In general, these secretory products of parasites constitute proteins, lipids and enzymes like proteinases and esterases which appear to assist in the invasion of the host tissue (Dzik, 2005) and also act as immunomodulatory molecules (Hewitson,Grainger,& Maizels,2009). Fernandez-Cabezudo et al (2017) demonstrated the activation of a neuro-immunological pathway in intestinal mucosal layer of the host to combat intestinalpathogens.

Both free-living and parasitic nematodes are found to have acetylcholine as an important neurotransmitter found in the neuromuscular system (Lee, 1996). Cholinesterase is an esterase

enzyme that terminates synaptic transmission of cholinergic synapses by hydrolyzing the neurotransmitter acetylcholine into choline and acetate (Colovic, Krstic, Lazarevic-Pasti, Bondzic,& Vasic, 2013).Two major types of cholinesterase exist in vertebrates. They are, namely, Acetylcholinesterase (AChE, EC 3.1.1.7) and Butyrylcholinesterase (BChE, EC 3.1.1.8) (Massoulie, 2002). All the invertebrates studied so far (nematodes and arthropods) have shown the presence of only AChE (Combes, Fedon, Grauso, Toutant,& Arpagaus, 2000; Kang et al, 2011; Kang, Lee, Koh, & Lee, 2011; Toutant, 1989). AChE found in invertebrates exhibits membrane-bound and secretory soluble forms (Hyne & Maher, 2003). Besides producing membrane-bound neuromuscular AChE (Devos & Dick, 1992),the gastrointestinal parasitic nematodes have been reported to synthesize a true acetylcholinesterase (Lee, 1996), which is secreted in soluble form intoits external environment (Podolska, Nadolna, Wąs, Gosz, & Szostakowska, 2012; Sanderson, 1972).

Ascaridia sp. is one of the most prevalent helminth parasites of birds (McDougald, 2011; Permin & Hansen, 1998), of which *Ascaridia galli*(Phylum- Nematoda, Class- Chromadorea, Order- Ascaridida, Family- Ascaridiidae, Common name- small intestinal roundworm)is a parasitic roundworm predominantly found in the intestine of domestic fowls (Yamaguti, 1961). *Ascaridia galli* inhabits the small intestine, causing ascaridiasis (Griffiths, 1978). In heavy infections, adult worms may migrate to the oviduct and can be found in the hens' egg (Jacobs, Hogsette, & Butcher, 2003). Sometimes, they can be seen in the feces of birds (Jacobs et al, 2003).

Since poultry is of high economic importance in India (Vetrivel&Chandrakumarmangalam, 2013), we designed our experiments on the domestic fowl, *Gallus gallus domesticus* as host species. *Ascaridia galli* infections are reported to severely diminish the absorption of nutrients, thereby suppressing growth rates of infected fowl (Das,Kaufmann, Abel, & Gauly, 2010). Haemorrhagic patches in duodenum, proliferation of mucus-secreting cellsand extensive damage of intestinal wall have also been observed (Ikeme, 1971).Therefore,helminth parasites may result in a substantial economic loss to the poultry industry (Kumar, Garg, Ram, Maurya, and Banerjee, 2015).

In the beginning, we briefly illustrated the life cycle of *Ascaridia galli* to give the readers a fundamental idea about how the infection is transmitted from fowl to fowl. The principal focus of our experiment was acetylcholinesterase (AChE) assay only.Previously, the kinetic properties of AChE in *Ascaridia galli* have been demonstrated by Gupta, Sanyal, and Duggal (1991). In the first phase of our study, the specific activity of AChE secreted by adult nematode*Ascaridia galli* collected from the intestine of *Gallus gallus domesticus* and the respective intestinal tissue of their site of attachment were determined. The second phase included the comparison of AChE activity in fresh parasite, intestinal tissue, E/S products and post-secretory parasite. We also discussed about the probable causes of the differences among the various levels of AChE activity with special reference to E/S product and post-secretory parasites.

II. Life Cycle of Ascaridia

Nematodes have either a species-specific, direct life cycle with bird-to-bird transmission by ingestion of infective eggs or larvae or have an indirect cycle that requires an intermediate host (eg. insects, snails, or slugs).

Ascaridia galli possess a direct life cycle through ingestion of infective embryonated eggs which possibly contain second or third larvae stage (Arauju & Bressan, 1997) as shown in Fig. 1. The eggs are then translocated to proventriculus where the larvae hatch within 24 hours (Ackert, 1923). After the larvae have hatched, they penetrate the mucosa of small intestine causing hemorrhage (Luna-Olivares et al, 2012). The prepatent period is minimum 28 days under temperate weather (Permin, Nansen, Bisgaard, & Frandsen, 1998).

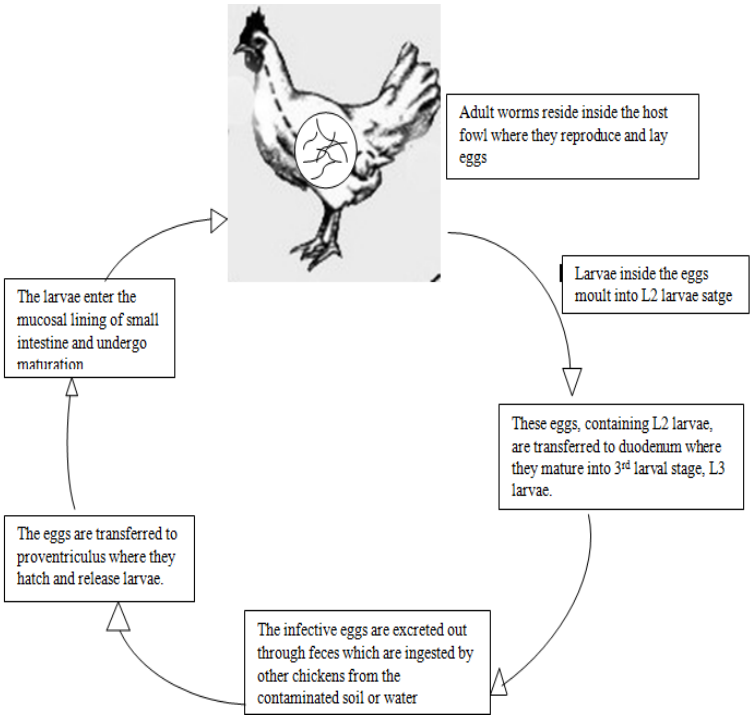

Fig.1. Life Cycle of *Ascaridia galli*

III. MaterialsandMethod

A. *Materials*

1) *Instruments*

- Glass Tissue Homogenizer
- Compact Cooling Centrifuge
- pH meter
- Eppendorf tubes of 1.5 ml
- Magnetic stirrer
- Microlitre pipettes
- Quartz Cuvette

- UV-VIS Spectrophotometer

2) Chemical reagents

- Sodium Chloride (NaCl)
- 4% Sodium Carbonate (Na_2CO_3) solution
- 4% Sodium Potassium Tartarate solution
- 2% Copper Sulphate ($CuSO_4$) solution
- Folin Ciocalteau reagent
- Sodium Phosphate buffer (pH 8.0 and 7.0)
- Ellman's reagent or Dithiobisnitrobenzoic acid (DTNB)
- Acetylthiocholine iodide

B. Method:

1) ParasiteCollection

A portion of the alimentary canal of host *Gallus gallus domesticus* were collected (both small intestine and large intestine) from the local commercial market. A total of 5hostintestinal samples were procured and were immediately brought to the laboratory in 0.9% physiological normal saline (9 g of sodium chloride dissolved in 1 liter of distilled water) solution. Intestines were dissected out longitudinally, and a total of 62 adult *Ascaridia galli* (75-105 mm in length) were recovered from the lumen of the hosts' intestine(Fig. 2). Of the 62recovered parasites, 31 fresh *A. galli*(6-7 parasites per host sample)were washed in normal saline. By following the method of Podolska and Nadolna (2014), they were kept for 48 hours at 4ºC in a separate small glass vial containing 10 ml of 0.9% physiological normal saline solution. This method was used to acquire the E/S product of the parasites.At the same time, rests of the fresh parasites were subjected to our immediate experiment.

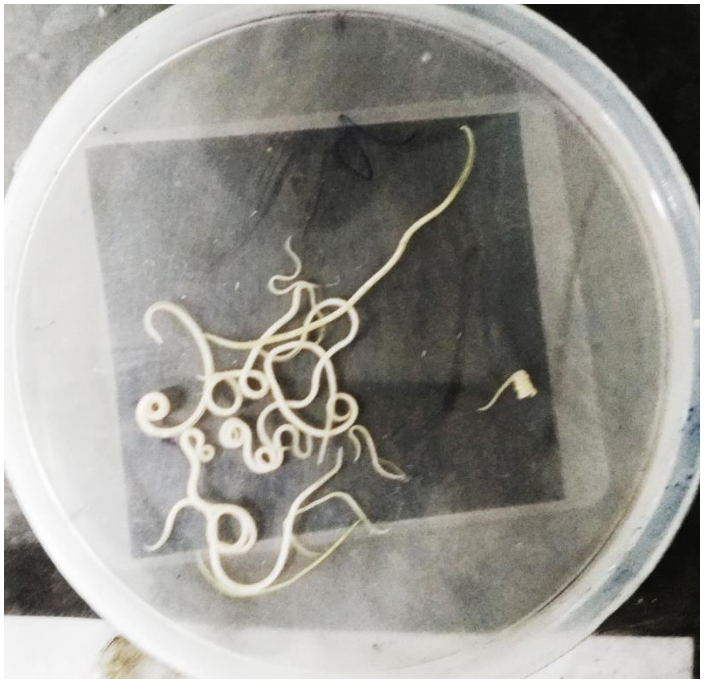

Fig. 2. *Ascaridia galli* found in intestine of *Gallus gallus domesticus*

2) Host tissuepreparation

After collection of the parasites, samples of the host's intestinal wall associated with the collected parasites were dissected out transversely into 5-6 small pieces and kept in normal saline at 4^0 C. Each piece was minced and prepared for homogenization.

3) Preparation ofhomogenates

The rest 31 fresh parasites and the minced tissues of intestine were homogenized separately by using a glass homogenizer. AChE activity was analyzed using total 6.262 g of host intestinal tissue and a total 5.084 g of parasites (each parasite weighs 35-130 mg).The homogenization for parasiteswas done in 15 ml of 0.1M sodium phosphate buffer (pH 8.0) at 4^0 C(5.13 ml of buffer per 1 g of parasite tissue)and that for host tissues was done in 12 ml of same buffer at 4^0 C (2 ml of buffer per 1 g of gut tissue). Then, the homogenates were centrifuged at 10,000 rpm for 5 minutes at 4^0C in a Compact Cooling Centrifuge. After that, the supernatants of both the samples were collected and were used to estimate AChE activity.

4) Preparation of post- secretory parasites and E/Sproduct

After 48 hours, the parasites kept for E/S product (Fig. 3)were collected from the cold saline solution as post-secretory parasites, washed in normal saline, homogenized and centrifuged following the similar procedure. Cold saline solution withoutparasites was then used for enzyme estimation as E/S product of the parasites.

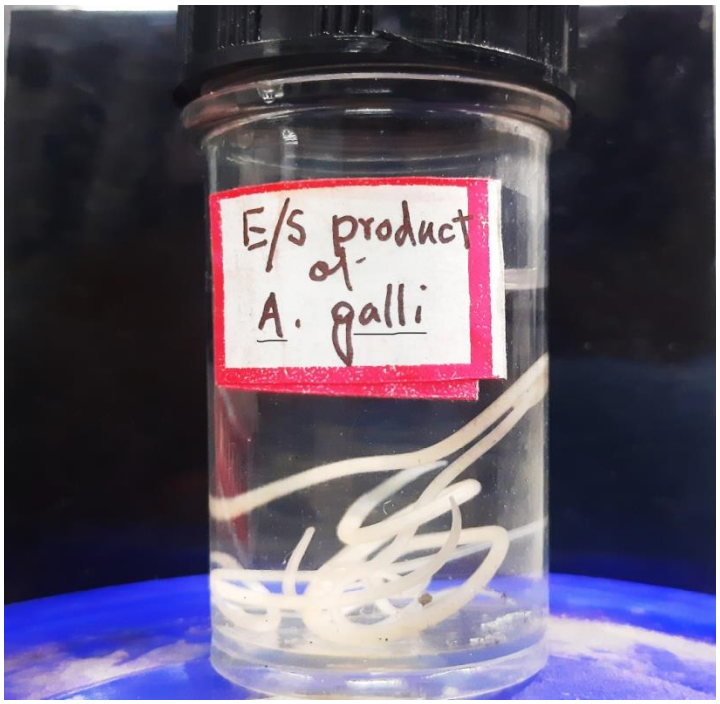

Fig. 3.*Ascaridia galli*in normal salineafter 48 hours

5) Determination of AChEactivity

Specific activity of AChE for each sample including the intestinal tissue, fresh parasite, E/S products and post-secretory parasites was measured by Ellman's reagent. The enzyme kinetics was observed at 412 nm using a UV-VIS

Spectrophotometer and a standard mixture(final volume is 3.12 ml) containing the supernatant, 0.1 M phosphate buffer (pH 8.0), 100 µl of Dithiobisnitrobenzoic acid (DTNB) and 20 µl of Acetylthiocholine iodide (Ellman, Courtney, Andrres, & Featherstone,1961). Prior to the enzyme activity measurement, the protein concentration was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) with the help of the Spectrophotometer, taking bovine serum albumin as the protein standard. The mean or average enzyme activity was measured for each sample.The whole experiment was repeated for seven times and the AChE activity was determined for four times in each sample to ensure reproducibility.

6) Statisticalanalysis

The AChE activity data were analyzed using the one-way analysis of variance (ANOVA) followed by the Tukey- Kramer test and expressed as the mean ± standard error mean (SEM). The differences of AChE activity between fresh parasites, intestinal tissue, E/S product and post secretory parasites were tested and were found to be significant at 5% level of significance ($p < 0.05$).

IV. Results

Despite having an undeveloped nervous system, high level of specific activity of AChE was observed in *A. galli* adult worm. The first phase of experiment was conducted immediately after extraction of the parasites. The mean or averageAChE activity of intestinal tissue of host *Gallus gallus domesticus* was 1.44±0.28 µmol/min/mg of protein. On the other hand, the mean AChE activity in fresh parasites was 3.89±0.32 µmol/min/mg of protein. The results indicated that the AChE level was higher in fresh parasitic nematodes than that in the host intestinal epithelium where the parasites resided (Fig. 4).As observed, AChE activity was 2.7 times higher in fresh parasites than in the host's intestinal tissue. This difference in enzyme activity was found to be statistically significant ($p<0.05$).

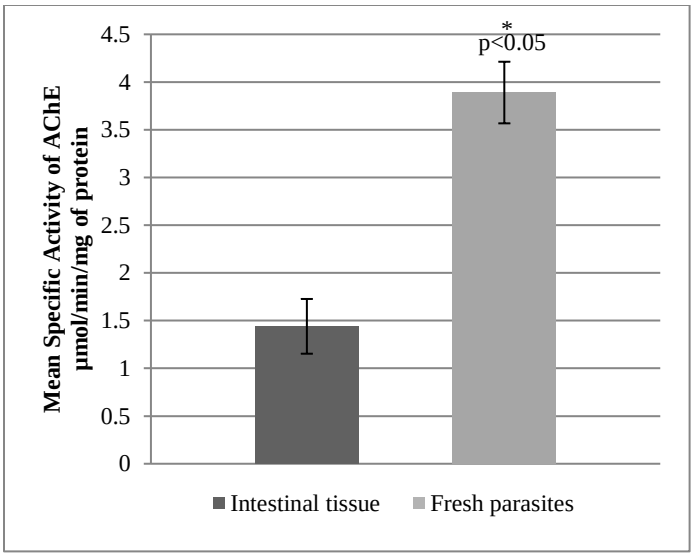

Fig. 4. Specific activity of AChE in intestinal tissue (SE: +/- 0.28) and fresh parasite (SE: +/- 0.32) in first experiment. AChE activity (µmol/min/mg of protein) is expressed as the mean +/- SEM and is measured immediately after the parasite extraction.

Later, enzyme activity was measured after 48 hours (Table I) and compared among the host's intestinal tissue, parasites, E/S product and in post-secretory parasites. The results of these measurements indicated that a mirror effect relationship existed between the enzyme activities of the host and its parasites. The highest AChE activity was found in post-secretory nematodes which was 6.73±0.34 µmol/min/mg of protein. On the contrary, enzyme activity in its ES products was recorded to be 3.68±0.28 µmol/min/mg of protein. The supernatant of the fresh parasitesshowed an AChE activityof 2.07±0.19 µmol/min/mg of protein. The enzyme activity of host's intestinal tissue was 1.16±0.11 µmol/min/mg of protein.In the second phase of the experiment, AChE activity was 3.25 times higher in post-secretory parasites and almost two times higher in E/S product than the enzyme activity recorded in fresh parasites (Fig. 5).The mean enzyme activity was considerably higher in post-secretory *A. galli* than that found in the supernatant of fresh *A. galli*. This variation in enzyme activity was found to be statistically significant ($p<0.05$) (Fig. 6).

Table I.Average specific activity of AChE after 48 hours (µmol/min/mg of protein±SEM)

Host Intestinal tissue	1.16±0.11
Fresh Parasites	2.07±0.19
E/S Product	3.68±0.28
Post-secretory parasites	6.73±0.34

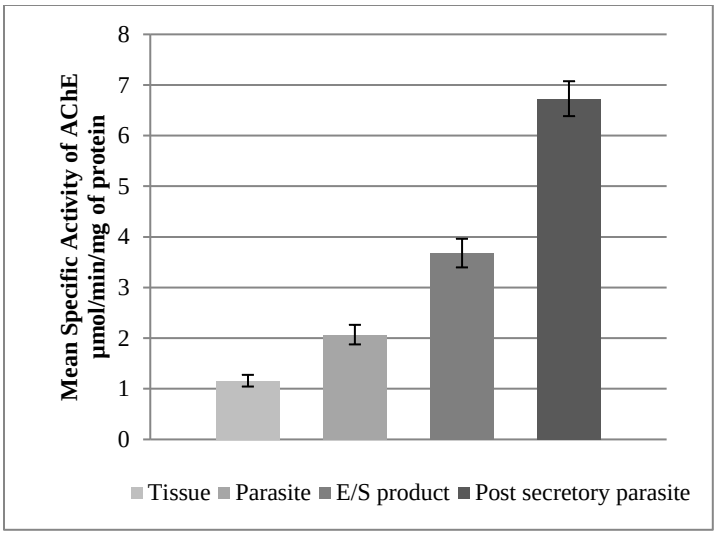

Fig. 5.Specific activity of AChE in host intestinal tissue (SE: +/- 0.11), fresh parasites (SE: +/- 0.19), E/S product (SE: +/- 0.28) and post-secretory parasites (SE: +/- 0.34). AChE activity is measured after 48 hours.

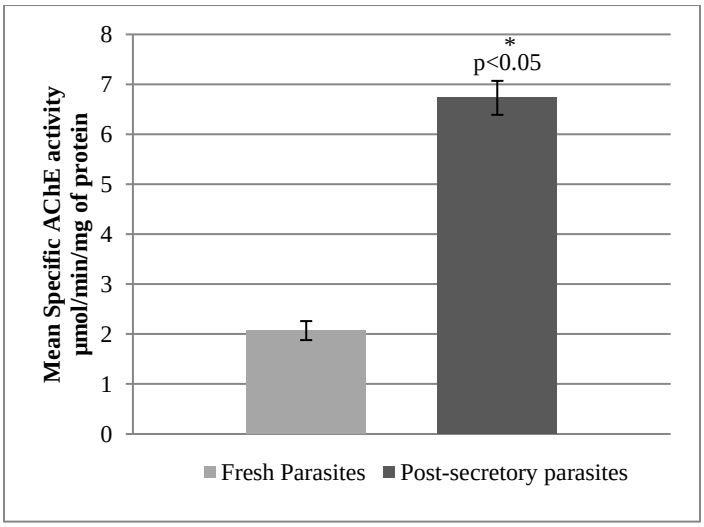

Fig. 6. The striking difference in AChE activity between fresh parasites (2.07±0.19 µmol/min/mg of protein)and post-secretory parasites (6.73±0.34µmol/min/mg of protein) is observed.

DISCUSSION

We predicted host-parasite interaction for one parasite species, that is, *Ascaridia galli* and studied the functional significance of this interaction through analysis of acetylcholinesterase (AChE) activity only. The 62 parasites were included in our research irrespective of their gender. Our present study demonstrates that the average AChE activity in the intestinal tissue of the fowl is inversely proportional to the average AChE activity in the extracts of fresh *A. galli* adult. The intestinal tissue of the host shows the lowest mean AChE activity as compared to the mean AChE activity of fresh parasites. Indeed, a similar pattern of enzyme activity relationship has been shown between the nematode parasite and its host tissue, for instance, between *Anisakis simplex* larvae and muscles of its host herring (Podolska & Nadolna, 2014).Earlier, the presence of acetylcholine (ACh) in both small and large intestines had been reported in animals and humans (Gautron et al, 2013; Jonsson, Norrgard, & Forsgren, 2007). ACh, secreted by enteric cholinergic neurons, stimulates enhanced production of mucus (Specian & Neutra, 1980) andsecretion of chloride (Cooke, 1984) to defend against pathogens. Therefore, we assume from our first phase of study that the *Ascaridia galli* parasites secreted AChE enzyme to combat against acetylcholine produced by the enteric cholinergic signaling system of the host. AChE, detected in the intestinal lumen of host in our study, is possibly a result of secretion byadult *Ascaridia galli* (Fig. 7). The outcome of our study could be supported by the work of Kennedy & Harnett (2001) and Selkirk, Lazari, & Matthews (2005) where it is well-documented that AChE, released by the nematode parasites, inhibits the process of chloride and mucus secretions by hydrolyzing acetylcholine. A recent study on host-parasite relationship by de Lange et al (2020) also strengthens our work. This experiment on *Taenia* larva hasdemonstrated that larvae of *Taenia crassiceps* release considerable amount of AChE in its excretory/secretory product which may be responsible for pathogenesis of neurocysticercosis (de Lange et al, 2020)

Some investigations have apparently suggested that AChE, secreted by gut dwelling nematodes, can modulate the immune system of the host (Pritchard, 1993; Rhoads, 1984). In *Nippostrongylus brasiliensis* adult, the enteric AChE inhibits the development of M2 macrophages and thus increases the chance of parasite survival (Vaux et al, 2016). Acetylcholine of hosts is capable of augmenting the activity of immunoglobulin molecules secreted by plasma cells (Brink et al,1994), release of various cytokines by neutrophils, inflammatory mediators by mast cells and lymphocyte-mediated cytotoxicity (Lee, 1996). Hence, AChE, secreted by parasite, may prevent all these anti-parasiticimmunological activities by breaking acetylcholine.

We found a considerable divergence in the AChE activity between host intestinal tissue and post secretory parasites. The AChE activity was almost three folds higher in E/S product than that in host tissue (Fig. 4). Moreover, the post sceretory parasites showed the highest AChE activity as compared to other samples when the parasites were kept at 4^0C. A study by Tinsley (1999) showed that parasites have evolved some crucial adaptation to survive in hostile surroundings. This may be the reason of higher activity of AChE in E/S product and in post secretory parasites in our study. Furthermore, AChE is secreted by parasitic worms to help them survive within host's body (de Lange et al, 2020). Therefore, we can presume that any inhospitable environment could trigger the parasites to release AChE as a strategy of survival under stressful condition (Fig. 7).

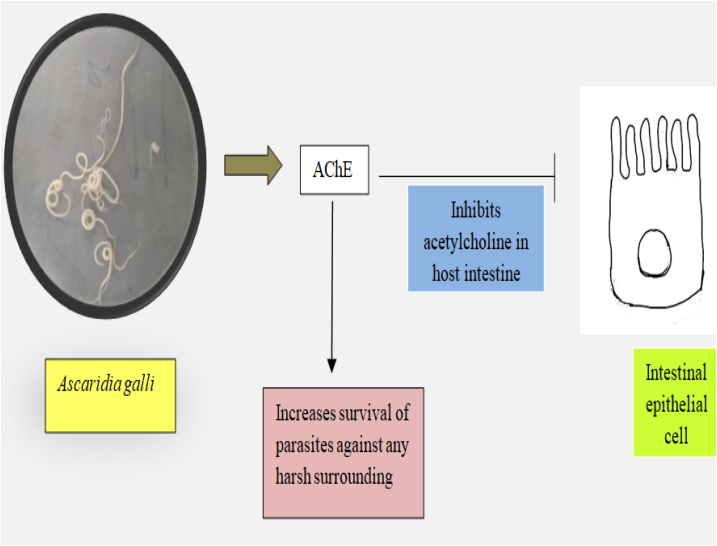

Fig. 7. Representative illustration of how AChE, released by *Ascaridia galli*, affects the host's intestinal ACh and facilitates the survival of parasites.

Conclusion and Future Direction

In our study, the analysis of acetylcholinesterase (AChE) activity suggests a critical interaction between parasites and the host system. In India, poultry establishes a major component of livestock sector with a poultry population of 729 million (National Action Plan for Egg & Poultry, 2022). Parasitic infection can seriously damage the economy of poultry industry. Therefore, it is essential to take steps against helminth parasites. Our experiment has successfully detected AChE as one of the molecules of the E/S product of nematode *Ascaridia galli*. Identification of these specific molecules in E/S product may lead to the development of neutralizing drugs against these intestinal roundworms. Our reports also reinforce the view that targeting AChE or any molecule of E/S product may be a suitable approach for vaccine development to prevent ascaridiasis or any helminthiasis. Monoclonal antibodies against the molecules of E/S product (as antigen) could be developed to treat any helminthiasis (Fig. 8).The above data of our small scale research is a preliminary data. Therefore, we are optimistic that our data would help in further analysis of AChE of *Ascaridia galli* in future.Since, a few studies have been conducted on *Ascaridia galli*, more investigations are recommended on this parasite as well as on its other E/S product molecules which could further broaden our understanding about the immunomodulation and could enlighten the path of potential therapies.

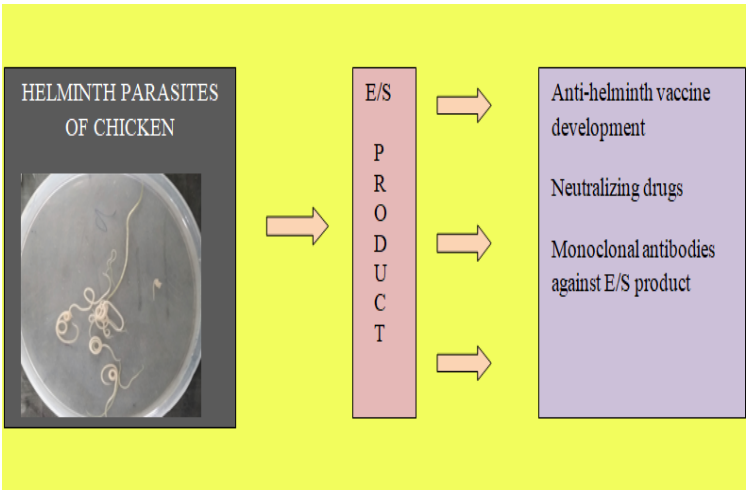

Fig. 8. Possibletreatments of ascaridiasis or any helminthiasis using E/S product as antigen.

Acknowledgment

The authors are thankful to the Principal, Dinabandhu Andrews College, Kolkata-700084, West Bengal for providing technical facilitiesand to the chicken seller of the local market for providing us intestine samples. Theauthors further would like to thank Mr. Subhabrata Datta for useful editing of the manuscript.

Authors Contribution

Sulagna Maity: Investigation,Enzyme activity analysis, Statistical Analysis, Performed literature research, Wrotefirst draft of the original manuscript, Correction (review & editing) of the manuscript.

Subhoshree Mondal: Investigation, Enzyme activity analysis and Wrotefirst draft of the manuscript.

Arup Kumar Ghosh: Methodology for collection of E/S product.

Ipsit Chakrabarti: Supervision,Conceptualization, Methodology, Validation, Collection of Resources, Project administration, Revision and Final correction of the manuscript.

All authors read and approved the finalmanuscript.

Declaration of Competing Interests

The authors have declared that no competing interests exist.

References

Ackert, J. E. (1923). On the habitat of *Ascaridia perspicillum* (Rud). *Anat. Rec.*, 26, 101-104.

Arauju, P.,& Bressan, C. R. (1977). Observations on the second moult of the larva of *Ascaridia galli*. *Ann. Parasitol. Hum. Comp.*, 52, 531-537.

Brink, P. R., Walcott, B., Roemer, E., Grine, E., Pastor, M., Christ, G. J.,&Cameron, R. H.(1994). Cholinergic modulation

of immunoglobulin secretion from avian plasma cells: the role of calcium. *J. Neuroimmunol*, 51, 113-121.

CDC Centers for Disease Control and Prevention. (2018).“CDC – Parasites - About parasites.” fromhttps://www.cdc.gov/parasites/about.html

Colovic, M. B., Krstic, D. Z., Lazarevic-Pasti, T. D., Bondzic, A. M.,& Vasic, V. M. (2013). Acetylcholinesterase inhibitors: Pharmacology and toxicology. *Curr Neuropharmacol.*, 11, 315-335.

Combes, D., Fedon, Y., Grauso, M., Toutant, J. P.,&Arpagaus, M. (2000). Four genes encode acetylcholinesterases in the nematodes *Caenorhabditiselegans* and *Caenorhabditisbriggsae*. cDNA sequences, genomic structures, mutations and in vivo expression. *J. Mol. Biol.*, 300, 727–742.

Cooke, H. J. (1984). Influence of enteric cholinergic neurons on mucosal transport in guinea pig ileum. *Am. J. Physiol.*, 246, G263-G267.

Das, G., Kaufmann, F., Abel, H., & Gauly, M. (2010). Effect of extra dietary lysine in Ascaridia galli-infected grower layers. *Vet Parasitol.*, 170(3-4), 238-243. https://doi.org/10.1016/j.vetpar.2010.02.026

de Lange, A., Prodjinotho, U.F., Tomes, H., Hagen, J., Jacobs, B.A., Smith, K., Horsnell, W., Sikasunge, C., Hockman, D., Selkirk, M.E., Prazeres da Costa, C., & Raimondo, J.V. (2020). Taenia larvae possess distinct acetylcholinesterase profiles with implications for host cholinergic signalling. *PLoS Negl Trop Dis.*, 14(12), e0008966. https://doi.org/10.1371/journal.pntd.0008966

DeVos, T.,& Dick, T. A.(1992). Characterization of cholinesterases from the parasitic nematode *Trichinella spiralis*. *Comp. Biochem. Physiol. C. Comp. Pharmacol. Toxicol.*,103, 129–134.

Dzik, J. M. (2005). Molecules released by helminth parasites involved in host colonization. *Acta Biochimica Polonica*., 53, 33-64.

Ellman, G. L., Courtney, K., Andrres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem.Pharmacol.*, 7, 88–95. https://doi.org/10.1016/0006- 2952(61)90145-9

Fernandez-Cabezudo, M. J., Al-Barazei, R., Bashir, G., Mohamed Yassir, A., Qureshi Mohammad, M., &Al-Ramadi, B. B. (2017). Cholinergic stimulation enhances intestinal antimicrobial activity and prevents systemic dissemination of pathogenic bacteria. *J. Immunol.*, 198 (1 Supplement), 151.18.

Gautron, L., Rutkowski, J. M., Burton, M. D., Wei, W., Wan, Y., & Elmquist, J. K. (2013). Neuronal and nonneuronal cholinergic structures in the mouse gastrointestinal tract and spleen. *J Comp Neurol*., 521(16), 3741-67.https://doi.org/10.1002/cne.23376

Griffiths, H. J.(1978). A Handbook of Veterinary Parasitology: Domestic Animals of North America (Minnesota, USA: University of Minnesota Press), 46-47.

Gupta, S., Sanyal, S. N., & Duggal, C. L. (1991). Study of the acetylcholinesterase activity of Ascaridia galli: kinetic properties and the effect of anthelmintics. *Acta Vet Hung*., 39(3-4), 165-174.

Hayunga, E. G. (1991). Morphological Adaptations of intestinal Helminths. *J. Parasitol.*, 77, 865-873.

Hewitson,J. P., Grainger, J. R.,& Maizels, R. M.(2009). Helminth immunoregulation: The role of parasite secreted proteins in modulating host immunity. *Molecular and BiochemicalParasitology.*, 167, 1-11.

Hussein, A., Harel, M., & Selkirk, M. (2002). A distinct family of acetylcholinesterasesis secreted by *Nippostrongylusbrasiliensis*. *Mol. Biochem. Parasitol.*, 123, 125–134.

Hyne, R. V., & Maher, W. A. (2003). Invertebrate biomarkers: links to toxicosis that predict population decline. *Ecotoxicol. Environ. Saf.*, 54, 366–374. https://doi.org/10.1016/s0147-6513(02)00119-7

Ikeme, M. M. (1971). Observations on the pathogenicity and pathology of *Ascaridia galli*. *Parasitology*, 63(2), 169-179.

Jacobs, R. D., Hogsette, J. A.,& Butcher, J. D.(2003). Nematode parasites of poultry (and where to find them). The Institute of Food and Agricultural Sciences (IFAS) series PS18 (University of Florida, USA).

Jönsson, M., Norrgård, O., &Forsgren, S. (2007). Presence of a marked nonneuronal cholinergic system in human colon: study of normal colon and colon in ulcerative colitis. *Inflamm Bowel Dis*, 13, 1347-56. https://doi.org/10.1002/ibd.20224

Kang, J. S., Lee, D. W., Choia, J. Y., Je, Y. H., Koh, Y. H., & Lee, S. H. (2011). Three acetylcholinesterases of the pinewood nematode, *Bursaphelenchusxylophilus*: insights into distinct physiological functions. *Mol. Biochem. Parasitol*., 175, 154–161.https://doi.org/10.1016/j.molbiopara.2010.11.005

Kang, J. S., Lee, D. W., Koh, Y. H., & Lee, S. H. (2011). A soluble acetylcholinesterase provides chemical defense against xenobiotics in the pinewood nematode. *PLoS ONE*, 6, e19063.

Kennedy, M. W., & Harnett, W. (Eds). (2001).Parasitic Nematodes - Molecular Biology Biochemistry and Immunology. Wallingford, UK: CABI Publishing.

Kumar, S., Garg, R., Ram, H., Maurya, P. S., & Banerjee, P. S. (2015). Gastrointestinal parasitic infections in chickens of upper gangetic plains of India with special reference to poultry coccidiosis. *Journal of parasitic diseases : official organ of the Indian Society for Parasitology*, 39(1), 22–26. https://doi.org/10.1007/s12639-013-0273-x

Lawrence, C.E., &Pritchard, D.I. (1993). Differential secretion of acetylcholinesterase andproteases during the development of *Heligmosomoidespolygyrus*. *Int. J. Parasitol.*, 23, 309–314.

Lee, D. L. (1996). Why do some nematode parasites of the alimentary tract secrete acetylcholinesterase? *Int. J. Parasitol.*,26, 499-508.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265-275.

Luna-Olivares, L. A., Ferdushy, T., Kyvsgaard, N.C., Nejsum, P., Thamsborg, S. M., Roepstorff, A., & Iburg, T. M. (2012). Localization of *Ascaridia galli*larvae in the jejunum of chickens 3 days post infection. *Vet Parasitol.*, 185(2-4), 186-193.https://doi.org/10.1016/j.vetpar.2011.10.025

Massoulie, J. (2002). The origin of the molecular diversity and functional anchoring of cholinesterases. *Neurosignals*, 11, 130-143.https://doi.org/10.1159/000065054

McDougald, L. R.(2011). Cestodes and trematodes.In Y.M. Saif, A.M. Fadly, J.R. Glisson, L.R. McDougald,L.K. Nolan,& D.E.Swayne (Eds.),Diseases of Poultry (pp. 961–972).Iowa, USA: Blackwell Publishing Company.

National Action Plan for Egg & Poultry for Doubling Farmers' Income by 2022, Ministry of Agriculture & Farmers' Welfare, Government of India, 2022.

Permin, A.,&Hansen, J.W.(1998). Epidemiology, Diagnosis and Control of Poultry Parasites (pp. 74-105, 111-118). Rome, Italy: Food and Agriculture Organisation.

Permin, A., Nansen, P., Bisgaard, M., & Frandsen, F. (1998). Investigations on the infection and transmission of *Ascaridia galli*in free range chickens kept at different stocking rates. *Avian Pathol.*, 27(4), 382-389.

Podolska, M., Nadolna, K., Wąs, A., Gosz, E., & Szostakowska, B. (2012). Acetylcholinesterase secretion by third-stage larvae of *Anisakis simplex* (Nematoda: Anisakidae) from Baltic herring. *Bull. Eur. Assoc. Fish Pathol.*, 32, 225–232.

Podolska, M.,& Nadolna, K. (2014). Acetylcholinesterase secreted by *Anisakis simplex* larvae [Nematoda: Anisakidae] parasitizing herring, *Clupea harengus*: an inverse relationship of enzyme activity in host-parasite system. *Parasitol.Res.*, 113, 2231-2238.https://doi.org/10.1007/s00436-014-3878-9

Pritchard, D.(1993).Why do some parasitic nematodes secrete acetylcholinesterase (AChE)? *International Journal for Parasitology*, 23, 549-550.https://doi.org/10.1016/0020-7519(93)90157-t

Rathaur, S., Robertson, B. D., Selkirk, M. E.,& Maizels, R. M.(1987). Secreted acetylcholinesterases from *Brugia malayi* adult and microfilarial parasites. *Mol. Biochem. Parasitol.*, 26, 257–65.

Rhoads,M. L. (1984). Secretory cholinesterases of nematodes: possible functions in the host-parasite relationship. *Trop. Veterinarian*,2, 3-10.

Sanderson,B. E. (1972). Release of cholinesterase by adult *Nippostrongylusbrasiliensisin vitro.Z. Parasitenkunde*., 40, 1–7.

Selkirk, M. E., Lazari, O., Hussein, A. S., & Matthews, J. B.(2005). Nematode acetylcholinesterases are encoded by multiple genes and perform non-overlapping functions. *Chem-Biol. Interact*., 157–158, 263–8.https://doi.org/10.1016/j.cbi.2005.10.039

Sharma, N., Hunt, P. W., Hine, B. C., McNally, J., Sharma, N. K., Iqbal, Z., Normant, C., Andronicos, N. M., Swick, R. A., & Ruhnke, I. (2018). Effect of an artificial *Ascaridia galli* infection on egg production, immune response, and liver lipid reserve of free-range laying hens. *Poult. Sci.*, 97(2), 494-502. https://doi.org/10.3382/ps/pex347

Specian, R. D.,&Neutra, M. R.(1980). Mechanism of rapid mucus secretion in goblet cells stimulated by acetylcholine. *J. Cell. Biol.*,85, 626-640.

Tinsley, R. C. (1999). Parasite adaptation to extreme conditions in a desert environment. *Parasitology*, 119, Supplement S1, S31-S56. https://doi.org/10.1017/S0031182000084638

Toutant, J. P. (1989). Insect acetylcholinesterase: catalytic properties, tissue distribution and molecular forms. *Prog. Neurobiol.*, 32, 423–446.

Vaux, R., Schnoeller, C., Berkachy, R., Roberts, L. B., Hagen, J., Gounaris, K., & Selkirk, M. E. (2016). Modulation of the Immune Response by Nematode Secreted Acetylcholinesterase revealed by Heterologous Expression in *Trypanosomamusculi*. *PLoS Pathog.*, 12, e1005998.

Vetrivel, S.C., & Chandrakumarmangalam, S. (2013). The Role of Poultry Industry in Indian Economy. *Revista Brasileira de Ciência Avícola*, 15, 287-293. https://doi.org/10.1590/S1516-635X2013000400001

Williamson, A. L., Lustigman, S., Oksov, Y., Deumic, V., Plieskatt, J., Mendez, S., Zhan, B., Bottazi, M. E., Hotez, P. J., & Loukas, A. (2006). *Ancylostomacaninum*MTP-1,an astacin-like metalloprotease secreted by infective hookworm larvae, isinvolved in tissue migration. *Infect Immun.*, 74, 961–967. https://doi.org/10.1128/IAI.74.2.961-967.2006

Yamaguti, S. (1961). Systema Helminthum. In:The nematodes of vertebrates (pp. 1261). New York and London: Interscience Publishers.

***