Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

EOSINOPHIL CATIONIC PROTEIN AND IRON STATUS IN PATIENTS INFECTED WITH *Enterobius vermicularis*

Esraa Wathah, Saleem Khteer Al-Hadrawy*

University of Kufa, Faculty of Science, Department of Biology, Iraq.

Received – September 22, 2021; Revision – October 12, 2021; Accepted – October 19, 2021

Available Online – October 30, 2021

DOI: [http://dx.doi.org/10.18006/2021.9\(5\).672.677](http://dx.doi.org/10.18006/2021.9(5).672.677)

KEYWORDS

Enterobiasis

Diarrhoea

Eosinophil

Kufa

Iron

ABSTRACT

The primary goal of this study was to determine the function of eosinophil cationic protein in iron status in patients infected with *Enterobius vermicularis*. For this, a total of 583 suspected patients and thirty healthy people of the same age who have visited the AL-Zahra maternity and pediatrics laboratory, AL-Hakeem hospital, AL-Sajad hospital AL-Fruit al-Awsat hospital, and AL-Munadira hospital in AL-Najaf province from July 2020 to June 2021 were screened. The presence of *E. vermicularis* eggs was estimated by using the saline wet mount technique from faeces samples of all respondents. Blood samples were collected from the 60 positive and 30 healthy control group and centrifuged at 3000 rpm for 5 minutes to separate serum, which was then collected in sterile tubes. Each serum sample was divided into three parts and stored in the deep freezer at -20°C until the serological test was performed. The level of ECP, iron and ferritin in enterobiasis patients was estimated from the isolated blood serum. According to the Manufacturer Company instructions, the concentration of two biomarkers (ECP, Ferritin) in serum samples was determined using the ELISA technique (Human reader, Germany). While the concentration of iron was evaluated using a colourimetric method. In comparison to the control group, the concentration of ECP was reported significantly higher ($P < 0.05$) in the *E. vermicularis* infected patients while the concentration of serum iron and ferritin was significantly decreased. The results of the current study can be concluded that *E. vermicularis* infection changes the serum ECP, iron, and ferritin concentration.

* Corresponding author

E-mail: saleem.alhadrawi@uokufa.edu.iq (Saleem Khteer Al-Hadrawy)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(<http://www.horizonpublisherindia.in/>).
All rights reserved.

All the articles published by [Journal of Experimental Biology and Agricultural Sciences](#) are licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](#) Based on a work at www.jebas.org.

1 Introduction

According to the World Health Organization, intestinal parasite infection is a major public health concern (Stoltzfus et al., 1997). According to Park et al. (2009), intestinal parasites infect more than 24% of people worldwide and harm approximately one billion people socially and economically. Parasites can invade the human intestine and cause anemia by impairing micronutrient absorption such as iron in the duodenum and jejunum that leads to iron deficiency anemia. Studies have been adopted on the tissue evidence such as eosinophils' effectiveness, the effectiveness of the eosinophils was tested in vitro and found to have the ability to kill parasites; the mechanism that eosinophils use against parasites is not fully understood but generally includes the production of antibodies. In most of the cases, it appears to involve antibody-induced release, complement-induced release, or both of toxic granule proteins and reactive oxygen intermediates by activation eosinophil; the acidity of the ability to kill parasites varies between the different stages of the life cycle of the parasite itself. Adults, children, and both sexes are all affected by *E. vermicularis* infections (Belete et al., 2019). Even though bowel disease is not so much harmful, but it is commonly seen as a parasitic infection, particularly in nations with a high infant mortality rate (Lohiya et al., 2000). Enterobiasis affects 200 million individuals worldwide (Brooker et al., 2006), and the disease's nature makes it easier to spread throughout families and educational institutions (Brooker et al., 2006). Pinworms can be found throughout the world, but they are most common in temperate regions (Kang et al., 2006). Pinworms are worms that exist worldwide and are difficult to control because they can easily be transmitted from person to person, these are mostly transferred through contaminated hands, direct finger and contact is usually an easy way to move between people with appropriate conditions such as primary schools, orphanages, refugee camps, and nurseries can also spread through the air by inhalation resulting in egg ingestion and infection (Burkhart & Burkhart, 2005). In the colon, adult worms are paired, after this, the pregnant females travel to the perianal area, where they deposit eggs, especially at night. Each female produces approximately 10,000 eggs (Ashford et al., 1988). Because the worms are also transmitted by autoinfection, transfer of infectious eggs by hand into the mouth from the site of itching, and the time between swallowing the infected eggs and laying eggs by adult females is 30 days, the female worms cause severe itching during their migration. Pinworms are diagnosed by placing a cellophane tape over the anus area in the early morning before defecating and it is microscopically examined (Garcia & Lynne, 2009). At severe infection conditions, worms can also be reported from the female genitalia, where they can cause diseases like vaginitis and damage to other organs, including the kidneys (Cateau et al., 2010). *E. vermicularis* cause a pelvic abscess to produce peritoneal inflammation in the peritoneal cavity (Das et al., 2001) while it is seen in urinary tracts of man's (Zahariou et al., 2007).

Because intestinal parasites use the source of food from their hosts, that's why these worms share various nutrients such as carbohydrates, minerals, vitamins, fats, and other essential energy sources of the host. These elements create serious physical ailments, so it is necessary to conduct routine parasite screening at regular intervals to detect the presence of infection with parasitic worms and to take appropriate treatment to eliminate the infection.

Minerals like magnesium, iron, copper, and zinc are essential for children's growth and development (Hesham et al., 2004). To achieve optimal growth and mental development in children, intestinal parasite infections must be detected and treated at an early stage (Pegelow et al., 1997). Olivares et al. (2003) recorded that trace elements influence metabolic pathways and the immunological response and suppress the occurrence of many diseases. Iron, copper, magnesium, and vitamin B12 are the most significant critical elements in the human body. Zinc is essential for immune system processes, and deficiency has reduced lymphocyte and thymus function. Zinc deficiency causes severe diarrhea in neonates due to its participation in immune system processes (Culha & Sangun, 2007). Copper is required to develop red blood cells, hemoglobin, iron absorption, and the action of several enzymes (Demirci et al., 2003). Copper and zinc are cofactors for cytosolic superoxide dismutase, and a reduction in the levels of these cofactors affects the functioning of this enzyme (Yousef et al., 2002; Demirci et al., 2003). In this manner, zinc is an important cofactor for many biological reactions.

Parasitic worms stimulate an intense Th2 response, which leads to the production of Interleukin-4 and Interleukin-5, which leads to the synthesis of immune globulin E (Al-Daoudy & Al-Bazzaz, 2020). In this manner, the presence of parasitic worms causes an increase in Th2, IgE, and eosinophilia cytokines. In response to this, helminths suppress the host's inflammatory response to Th2 and boost T and B cells (Rashad & Aljanaby, 2021). On the other hand, macrophages create cytokines including IL-10 and TGF β , as well as IgG4 antibodies that block IgE (McSorley & Maizels, 2012; Abd-Aljabar & Aljanaby, 2021). Eosinophiles play an essential role in the immune response. The eosinophile and eosinophil granular products such as Eosin cationic protein (ECP), eosinophil-derived neurotoxin (EDN), eosinophil peroxidase (EPO) were found to be efficient in worm management under in vitro conditions (Taliaferro & Sarles, 1939). The role of eosinophils in the organism's body is more complicated. Due to changes in the organism or the organism's immune response, many studies have been conducted about the role of eosinophils against different types of parasites (David et al., 1980). This study aimed to estimate the levels of serum eosinophil cationic protein (ECP), ferritin, and iron in the children (before 12 years old) suffering from enterobiasis disease, and to establish a relation between the level of these biomarkers and *E. vermicularis* infection.

2 Materials and Methods

The occurrence of acute anal itching, especially at night (the most prevalent), as well as apparent clinical indications such as irritability, lack of appetite, abdominal pain, urinary tract infections, yellowing of the face, and weight loss, are the important symptoms of *E. vermicularis* infection (Garcia & Lynne, 2009). The current study was conducted on 583 suspected patients from July 2020 to June 2021; stool samples were collected from these suspected patients and diagnosed by light microscope using wet mount preparation for the detection of adult pinworms or eggs at the microbiological laboratory, department of biology, college of science, Al-Kufa University. To detect the presence of *E. vermicularis* eggs, all faeces samples were examined using the saline wet mount technique, which involved placing a drop of saline (0.9 percent) on a glass slide. For this, a small amount of faeces samples were spread over the glass slide, mixed with a saline drop, covered with a coverslip, and observed at 100 X and 400 X, magnifications under a light microscope (Al-Hadraawy et al., 2019).

2.1 Serum biomarker detection

For the immunological analysis, 5 ml blood samples were taken from 60 *E. vermicularis* positive individuals and 30 healthy children (controls) and maintained at room temperature for 30 minutes (Al-labban et al., 2021; Azeez et al., 2021). The blood samples were centrifuged at 3000 rpm for 5 minutes and serum sample was collected in sterile tubes. Each serum sample was divided into three parts and stored in the deep freezer at -20°C until the serological test was performed. According to the Manufacturer's Company, the concentration of biomarkers (ECP in Ferritin) in serum was determined using the ELISA technique (Human reader, Germany). BTLAB provided these biomarkers units with Cat.No. E1391Hu and Cat.No. E1702Hu, respectively, while iron concentration was quantified using a colourimetric method as per the standard protocol (Al-Hadraawy et al., 2016; Al-Hadraawy et al., 2019; Mohy et al., 2019).

2.2 Statistical analysis

The healthy and control groups were compared using Graph-pad prism version 10-computer software (T-test). A statistically significant P-value of less than 0.05 was used (Al-Hadraawy et al., 2016).

3 Results and Discussion

In the current investigation, the concentration of (ECP) in *E. vermicularis* infected males and females was significantly higher ($P=0.05$) than in the control group. As shown in table 1, the concentration of ECP was reported 32.48ng/ml and 34.42ng/ml in males and females respectively while in the case of total patients

this value was reported 33.35ng/ml. While iron and ferritin concentration was significantly lower ($P=0.05$) in *E. vermicularis* infected males and females as compared to the control. In the case of total patients, *E. vermicularis* infected patients have 62.23 ($\mu\text{g/ml}$) and 72.16 (ng/ml) iron and ferritin concentration while this value was reported 86.63 ($\mu\text{g/ml}$) and 79.19 (ng/ml) in control respectively. Overall *E. vermicularis* influenced the body's immune system and enhance the secretion of ECP.

Table 1 Effect of *E. vermicularis* infection on the selected biological markers

Respondents	Biological Markers		
	Iron ($\mu\text{g/ml}$)	Eosinophil Cationic Protein(ng/ml)	Ferritin (ng/ml)
Male (Patients)	62.29 $\pm$ 0.82	32.48 $\pm$ 1.16	72.52 $\pm$ 0.69
Male (Control)	87.33 $\pm$ 1.05	1.65 $\pm$ 0.59	78.89 $\pm$ 1.76
Female (Patients)	62.17 $\pm$ 0.84	34.42 $\pm$ 0.81	71.87 $\pm$ 1.15
Female (Control)	85.93 $\pm$ 1.28	1.79 $\pm$ 0.04	79.95 $\pm$ 1.35
Total Patients	62.23 $\pm$ 0.58	33.35 $\pm$ 0.83	72.16 $\pm$ 0.67
Total Control	86.63 $\pm$ 0.82	10.70 $\pm$ 0.39	79.19 $\pm$ 1.09

Results of this study demonstrate that males and females infected with *E. vermicularis* have significantly higher concentrations of ECP compared to the control group. This outcome could be due to worm infection, which triggers an immune response that leads to a sequence of cellular and molecular interactions with mast cells, which are involved in the stimulation of immunoglobulin E (Bethony et al., 2006). This increasing number of eosinophils enhances the production of leukocytes and pellets which kills or expels parasitic worms from the body (Reimert et al., 1991; Reimert et al., 2006). This might be a possible explanation of higher ECP levels in people suffering from *E. vermicularis* infection as compared to healthy people. Among the proteins secreted by eosinophils ECP, EDN, and EPO are the most common. Among these, EPO might play an important role in the parasite defense mechanism (Hamann et al., 1990). These results are in agreement with the findings of Tischendorf et al. (2000). These researchers reported a higher value of ECP with median levels ranging from 46 to 98 g/l in persons infected with parasites. These results are also confirmed by the findings of Amoani et al. (2019) and Hassan et al. (2002), those who reported higher ECP levels in hookworm and helminthic infected participants as compared to the healthy individuals respectively. Similarly, the findings of Tischendorf et al. (2000) refer to EDN's high value in parasite-infected individuals. At the same time, the results of the current study are contradictory to the findings of Amoani et al. (2019) those who suggested that serum ECP levels do not rise

during malaria infection. ECP and EDN/EPX levels were reported to be higher in filariae and schistosome infections linked to eosinophilia (Tischendorf et al., 1996; Iskra et al., 2020).

Compared to the control group, the ferritin concentration in males and females having *E. vermicularis* infections was considerably lower. This might be because of worm infections because Osazuwa et al. (2011) suggested that worm infection is more sensitive in identifying anaemia and was linked to helminth infection in the intestine. The findings of this study accord with those of Adebara et al. (2011) those who identified a link between intestinal helminthiasis and serum ferritin levels in Nigerian school children and observed that ferritin levels were lower in hookworm infected children. Jafer et al. (2015) also reported that ferritin levels increased considerably after hookworm therapy. Similarly, Bhargava et al. (2003) found that serum ferritin levels were low in children infected with intestinal parasites such as *Ascaris* and *Trichuris*. Le et al. (2007) and Bayoumy et al. (2018) explained the substantial relationship between serum ferritin values and parasites and suggested that patients with parasitic illness have low serum ferritin levels.

The results demonstrate that iron concentrations in males and females infected by *E. vermicularis* have significantly lower than in the control group; this could be because infections hindered iron absorption from the gastrointestinal tract (Stoltzfus et al., 1997; Li et al., 2007). These findings are comparable to the results of Al-Daoudy & Al-Bazzaz (2020) those who found that the mean blood total protein and serum iron levels were considerably lower in the enterobiasis-positive groups than the enterobiasis-negative group's. Olsen et al. (1998) reported that the presence of intestinal parasites generally accompanied the emergence of iron deficiency anaemia. Similarly, In a study by Kuvibidila et al. (1995) children, with parasite and protozoa infections had a higher rate of iron deficiency anaemia (IDA) than those who were not affected. An essential link between parasitic intestinal infection and iron deficiency anaemia among school children in rural Vietnam was discovered in Southeast Asia. Results of this study are contradictory to the findings of Da Silva et al. (2016); Darlan et al. (2018) and Khazaaal et al. (2020) those who could not discover an association between serum iron levels and intestinal parasite infection. Similarly, Ngui et al. (2014) could not discover any link between anaemia and parasitic intestinal illness in elementary school pupils in a study conducted in Brazil.

Conclusions

Results of the study can be concluded that *E. vermicularis* infection has a significant effect on the serum ECP, ferritin, and iron concentration. Higher ECP concentration might be an attempt of the human body against worm infection. While the lower iron concentrations are showing malfunctioning of the intestine.

References

- Abd-Aljabar EM, Aljanaby AAJ (2021) Role of macrophage migration inhibitory factor, cluster of differentiation 19 and interleukin 23 in individuals infected with *Salmonella typhi*. *Journal of Experimental Biology and Agricultural Sciences* 9(3):394 – 400. DOI: [http://dx.doi.org/10.18006/2021.9\(3\).394.400](http://dx.doi.org/10.18006/2021.9(3).394.400)
- Adebara OV, Ernest SK, Ojuawo (2011) Association between intestinal helminthiasis and serum ferritin levels among school children. *Open Journal of Pediatrics* (1):12-16.
- Al-Daoudy AAK, Al-Bazzaz EN (2020) Impact of *Enterobius vermicularis* infection on biochemical parameters in the blood of children in Erbil Province, Iraq. *BMC Infectious Diseases* 20:1-6.
- Al-Hadraawy SK, Al-ghurabi ME, Al-musawi MM, Alzeyadi M (2016) Ghrelin and melatonin as biomarkers in patients with giardiasis. *Biotechnology & Biotechnological Equipment* 30(3): 553-557.
- Al-Hadraawy SK, AL-Shebly FM, Abood AH, Kadhim NJ (2019) Correlation study between biological markers in patients with *Entamoeba histolytica* parasite. *Biochemical and Cellular Archives* 19(1): 125–129.
- Al-labban HMY, Al-luhaiby AI, Salih NS, Yahya WI, Aljanaby AAJ (2021) Study of azo compounds derived from p-cresol ligand: synthesis, characterization and biological activity. *Journal of Experimental Biology and Agricultural Sciences*, 9(3): 401 – 406.
- Amoani B, Adu B, Frempong MT, Sarkodie-Addo T, Nuvor SV, Wilson MD, Gyan B (2019) Levels of serum eosinophil cationic protein are associated with hookworm infection and intensity in endemic communities in Ghana. *PloS one* 14(9): e0222382.
- Ashford RW, Hart CA, Williams RG (1988) *Enterobius vermicularis* infection in a children's ward. *Journal of Hospital Infection* 12(3):221-224.
- Azeez MR, Aljanaby AAJ, Öcal IÇ (2021) Phenotypic and genotypic characterization of five antimicrobial resistance genes associated with *Klebsiella pneumoniae* isolated from burn infection patients. *Journal of Experimental Biology and Agricultural Sciences* 9(3):378 – 387. DOI: [http://dx.doi.org/10.18006/2021.9\(3\).378.387](http://dx.doi.org/10.18006/2021.9(3).378.387).
- Bayoumy A, Hassan KA, Geneidy MR, Metawea AG (2018) Impact of intestinal parasites on hematological parameters among school children in Gharbia Governorate, Egypt. *Journal of the Egyptian Society of Parasitology* 48(1): 157-164.

- Belete Y, Asrat D, Woldeamanuel Y, Yihnew G, Gize A (2019) Bacterial Profile And Antibiotic Susceptibility Pattern Of Urinary Tract Infection Among Children Attending Felege Hiwot Referral Hospital, Bahir Dar, Northwest Ethiopia. *Infection and Drug Resistance* 12:3575-3583. doi: 10.2147/IDR.S217574. eCollection 2019.
- Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ (2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet* 367(9521): 1521-1532.
- Bhargava A, Jukes M, Lambo JCM, Kihamia W, Nokes LC, Drake L, Bundy L (2003) Anthelmintic treatment improves the hemoglobin and serum ferritin concentrations of Tanzanian school children. *Food and Nutrition Bulletin* 24 (4): <https://doi.org/10.1177/156482650302400403>.
- Brooker S, Clements AC, Bundy DA (2006) Global epidemiology, ecology and control of soil-transmitted helminth infections. *Advances in Parasitology* 62: 221-261.
- Burkhart CN, Burkhart CG (2005) Assessment of frequency, transmission, and genitourinary complications of enterobiasis (pinworms). *International Journal of Dermatology* 44(10): 837-840.
- Cateau E, Yacoub M, Tavilien C, Becq-Giraudon B, Rodier MH (2010) *Enterobius vermicularis* in the kidney: An unusual location. *Journal of Medical Microbiology* 59(7): 860-861.
- Culha G, Sangun MK (2007) Serum levels of zinc, copper, iron, cobalt, magnesium, and selenium elements in children diagnosed with *Giardia intestinalis* and *Enterobiosis vermicularis* in Hatay, Turkey. *Biological Trace Element Research* 118(1): 21-26.
- Da Silva JV, Fontes LG, dos Santos RV (2016) Factors associated with gastrointestinal parasitic infections among young population in Northeast Brazil. *Canadian Journal of Infectious Diseases and Medical Microbiology* Article ID 6239434 <https://doi.org/10.1155/2016/6239434>.
- Darlan DM, Ananda FR, Sari MI, Arrasyid NK, Sari DI (2018) Iron deficiency anemia and intestinal parasitic infection in school-age children in Medan IOP Conference Series: Earth and Environmental Science 125: 012059
- Das DK, Pathan SK, Hira PR, Madda JP, Hasaniah WF, Juma TH (2001) Pelvic abscess from *Enterobius vermicularis*. Report of a case with cytologic detection of eggs and worms. *Acta cytologica* 45(3): 425-429.
- David JR, Vadas MA, Butterworth AE, de Brito PA, Carvalho EM, David RA, Andrade ZA (1980) Enhanced helminthotoxic capacity of eosinophils from patients with eosinophilia. *New England Journal of Medicine* 303(20): 1147-1152.
- Demirci M, Delibas N, Altuntas I, Oktem F, Yonden Z (2003) Serum iron, zinc and copper levels and lipid peroxidation in children with chronic giardiasis. *Journal of Health, Population and Nutrition* 21(1):72-75.
- Garcia, Lynne S (2009) Practical guide to diagnostic parasitology. American Society for Microbiology, Pp. 246-247. ISBN 978-1-55581-154-9.
- Hamann KJ, Gleich GJ, Checkel JL, Loegering DA, McCall JW, Barker RL (1990) In vitro killing of microfilariae of *Brugia pahangi* and *Brugia malayi* by eosinophil granule proteins. *The Journal of Immunology* 144(8): 3166-3173.
- Hassan M, El-Motaim M, Mattar M, Afify H, Badrawy ES (2002) Assessing the morbidity of schistosomiasis by measuring eosinophil cationic protein in serum. *Journal of the Egyptian Society of Parasitology* 32(2):517-24.
- Hesham MS, Edariah AB, Norhayati M (2004) Intestinal parasitic infections and micronutrient deficiency: a review. *The Medical Journal of Malaysia* 59(2): 284-293.
- Iskra GR, Iskren TK, Nina DT (2020) Study of eosinophil cationic protein serum levels in patients with toxocariasis Eleonara Marinova Kanevaa, Rumen Nenkov. *Iberoamerican Journal of Medicine* 03 178-182.
- Jafer EH, AL Jabbar JA, Attawi AB, Mohammad TK (2015) Relations between Iron Deficiency Anemia and Anemia from Hookworms Parasites The Iraqi Journal of Veterinary Medicine 39(2): 66-71.
- Kang S, Jeon HK, Eom KS, Park JK (2006) Egg positive rate of *Enterobius vermicularis* among preschool children in Cheongju, Chungcheongbuk-do, Korea. *The Korean Journal of Parasitology* 44(3): 247.
- Khazaal RM, Al-hadraawy SK, Hussein KR (2020) Prevalence of *Enterobius vermicularis* among pre-school age and school-age children in Thi-Qar province southern Iraq. *International Journal of Pharmaceutical Research*, Pp. 857-864. <https://doi.org/10.31838/ijpr/2020.SP1.134>.
- Kuvibidila S, James MA, Warriar RP, Yu RP, Ode D, Tshetu KA (1995) Soluble transferrin receptor as an index of iron status in Zairian children with malaria. *Journal of Tropical Medicine and Hygiene* 98:373-8.

- Le H T, Brouwer ID, Verhoef H, Nguyen KC, Kok FJ (2007) Anemia and intestinal parasite infection in school children in rural Vietnam. *Asia Pacific Journal of Clinical Nutrition* 16(4): 716-723.
- Lohiya GS, Tan-Figueroa L, Crinella FM, Lohiya S (2000) Epidemiology and control of enterobiasis in a developmental center. *Western Journal of Medicine* 172(5): 305.
- McSorley HJ, Maizels RM (2012) Helminth infections and host immune regulation. *Clinical Microbiology Reviews* 25(4): 585-608.
- Mohy AA, Aljanaby AAJ, Al-Hadraawy SK (2019) Evaluation of Eosinophilic Cationic Protein and Some Immunological Markers in Patients Infected with Scabies. *Journal of Pure and Applied Microbiology* 13(3):1743-1737.
- Ngui R, Ravindran S, Ong DBL, Chow TK, Low KP, Nureena ZS, Mahmud R (2014) *Enterobius vermicularis* saplingit's seen in the setting of ectopic pregnancy in a Malaysian patient. *Journal of Clinical Microbiology* 52(9): 3468-3470.
- Olivares JL, Fernandez R, Fleta J, Rodriguez G, Clavel A (2003) Serum mineral levels in children with intestinal parasitic infection. *Digestive Diseases* 21(3):258-261.
- Olsen A, Magnussen P, Ouma JH, Andreassen J, Friis H (1998) The contribution of hookworm and other parasitic infections to haemoglobin and iron status among children and adults in western Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 92(6): 643-649.
- Osazuwa F, Ayo OM, Imade PA (2011) Significant association between intestinal helminth infection and anaemia burden in children in rural communities of Edo state, Nigeria. *North American Journal of Medical Sciences* 3(1) 30-34.
- Park CW, Kim JS, Joo HS, Kim J (2009) A human case of *Clinostomum complanatum* infection in Korea. *The Korean Journal of Parasitology* 47(4): 401.
- Pegelow K, Gross R, Pietrzik K, Lukito W, Richards AL, Fryauff DJ (1997) Parasitological and nutritional situation of school children in the Sukaraja district, West Java, Indonesia. *The Southeast Asian Journal of Tropical Medicine and Public Health* 28(1): 173-190.
- Rashad JMBM, Aljanaby AAJ (2021) Role of interleukin-2, interleukin-4 and cluster of differentiation-22 as an immune markers in individuals infected with helicobacter pylori. *Journal of Experimental Biology and Agricultural Sciences* 9(3): 388 – 393. DOI: [http://dx.doi.org/10.18006/2021.9\(3\).388.393](http://dx.doi.org/10.18006/2021.9(3).388.393)
- Reimert CM, Fitzsimmons CM, Joseph S, Mwatha JK, Jones FM, Kimani G, Vennervald BJ (2006) Eosinophil activity in *Schistosoma mansoni* infections in vivo and in vitro in relation to plasma cytokine profile pre-and posttreatment with praziquantel. *Clinical and Vaccine Immunology* 13(5): 584-593.
- Reimert CM, Venge P, Kharazmi A, Bendtzen K (1991) Detection of eosinophil cationic protein (ECP) by an enzyme-linked immunosorbent assay. *Journal of Immunological Methods* 138(2): 285-290.
- Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M, Savioli L (1997) Epidemiology of iron deficiency anemia in Zanzibari school children: the importance of hookworms. *The American journal of clinical nutrition* 65(1): 153-159.
- Taliaferro WH, Sarles MP (1939) The cellular reactions in the skin, lungs and intestine of normal and immune rats after infection with *Nippostrongylus muris*. *The Journal of Infectious Diseases* 64(2): 157-192.
- Tischendorf F, Brattig N, Buttner D, Pieper A, Lintzel M (1996) Serum levels of eosinophil cationic protein, eosinophil-derived neurotoxin and myeloperoxidase in infections with filariae and schistosomes. *Actatropica* 62(3):171-82.
- Tischendorf FW, Brattig NW, Lintzel M, Buettner DW, Burchard GD, Bork K, Müller M (2000) Eosinophil granule proteins in serum and urine of patients with helminth infections and atopic dermatitis. *Tropical Medicine & International Health* 5(12): 898-905.
- Yousef MI, El-Hendy HA, El-Demerdash FM, Elagamy EI (2002) Dietary zinc deficiency induced-changes in the activity of enzymes and the levels of free radicals, lipids and protein electrophoretic behavior in growing rats. *Toxicology* 175(1-3): 223-34.
- Zahariou A, Karamouti M, Papaioannou P (2007) *Enterobius vermicularis* in the male urinary tract: a case report. *Journal of Medical Case Reports* 1(1): 1-3.