

Haematological Parameters and Cellular Immune Response Associated with Administration of Polyvalent *Schistosomamansoni* Vaccine in Mice

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Abstract: *Schistosomiasis is one of the most tropical neglected diseases (NTDs), which have very complex antigenically different life stages. So that studying the effect of each antigen on cellular immune response is required. Three schistosomal antigens were used CAP, SEA and SWAP separately in vivo. Blood parameters and lymphocytes blastogenesis were evaluated. The most changes were reported in WBCs and differential cells with maximum immune response after 33 dpv in CAP and 47 dpv in SEA and SWAP. These changes reflected the defense and cellular immune response for each antigen.*

Keywords: CAP, SEA, SWAP, schistosomiasis, blastogenesis

1. Introduction

Schistosomiasis or bilharzia is a vector-borne parasitic disease that caused by flatworm trematoda of the genus *Schistosoma* [1]. The importance of Schistosomiasis was in terms of its public health and socioeconomic impact after malaria in many developing countries of the tropics [2]. The overall annual mortality rate might exceed 200,000 people in Africa as a result of different complications of urinary and intestinal schistosomiasis [3]. *Schistosomamansoni* causes intestinal schistosomiasis and infects about 100 million people in tropical regions [4]. The focusing on the development of vaccine against schistosomiasis together with chemotherapy would have a great impact in the disease control and elimination [5]. But the ability of the parasite to escape from the host immune system and its complex life cycle make the development of vaccine against schistosomiasis a different task to achieve [5]. Schistosomes have a complex life cycle which is important for understanding the immunology of the host-pathogen interplay [6]. They are antigenically very complex organisms, and stage-specific antigens are found among schistosome, cercaria, egg or adult worms [7, 8].

Blood is the routinely tissue which used for many comparative studies. It has been demonstrated to be good indicators of immune system [9, 10, 11]. The lymphocyte transformation has been used to evaluate one aspect of cell immunity in patients with schistosomiasis, leprosy, syphilis, paracoccidiodomycosis, and other infectious diseases [12]. Mussatti et al. [13] and Pagnano et al. [14], however, found differences in response between lymphocytes from both patients and donors when cultured in autologous or homologous plasma. In the present study, several procedures were used to determine the changes of immunological biomarkers for different types of schistosomal antigens that can be identified using blood parameters and lymphocyte blast-transformation with correlation between antibodies produced by different soluble antigens and serum factors. Finding such biomarkers would improve the antigenicity of

each type. The cell mediation of immunity to *Schistosomamansoni* remains a controversial issue [15] which demonstrated that the infected individual is able to mediate cellular reactions like blastogenesis to polyclonal mitogens and soluble antigens during the different phases of the disease. The infected individuals are able to mediate cellular reactions like blastogenesis to polyclonal soluble antigens during different phases of the disease and such functions have been reported and related with parasitic antigens [16, 17, 18, 19].

2. Materials and Methods

2.1. Experimental animals and vaccination design

A total number of 108 pathogen-free Swiss albino mice (25-38 g) were used. Animals were fed on standard chow, supplied with water at housing laboratory in the faculty of Veterinary Medicine, Assiut University, Egypt. The test groups received subcutaneous injections separately with cercarial antigen prepared (CAP), soluble egg antigen (SEA) at 0, 3, 11, and 25 days post first vaccination (dpfv), and with soluble worm antigen prepared (SWAP) at 0, 3, 11, 25, 28, 30, and 37 dpfv. The corresponding control group of each antigen received the same injections of adjuvant in the same time; the negative control remained without any injections. In order to further investigate about the titer of antibodies by each antigen in vaccinated mice, sera from vaccinated mice were collected at 7, 20, 33, and 47 days post vaccination (dpv) schedule.

2.2. Schistosomal antigens preparation:

Cercarial antigen preparation (CAP), soluble worm antigen preparation (SWAP) and soluble egg antigen (SEA) were prepared at the *Schistosoma* Biological Supply Program (SBSP) at Theodor Bilharzia Research Institute (TBRI). CAP was prepared according to the method of Carter and Colley [20], SWAP was prepared according to the method of Salih et al. [21] and SEA was prepared according to Boros and

Warren [22]. Freund's adjuvant (Adj) {Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA)} was obtained from Sigma Chemical Co., St Louis, Mo, USA and emulsified in phosphate buffered saline (PBS) at a ratio of 2:1 (v/v).

2.3. Hematological Parameters

Blood samples were collected by penetrating the retro-orbital plexus/ sinus with a heparin- treated glass capillary tube from individual mice and separate in tubes with anticoagulant for blood parameters measurements. The blood parameters were measured using the automated hematological analyzer (EXIGO Veterinary Analyzer). The percentage of lymphocyte blastogenesis were counted using blood smears stained with Giemsa stain by counting 100 cells in each smear with 3 replicates for each group.

3. Results

3.1. Effect of Different Antigens Onhaematological Parameters:

The physical and haematological parameters of the three vaccinated groups and control group showed in Table (2, 3, 4 and 5) and Figs.(2, 3, 4).

In CAP-vaccinated group, the trend on the changes in the total white blood cells and red blood cells was parallel to the results of antibodies production (Table 2, 3, 4 and 5; Figs. 2) which showed by significant increase ($P < 0.05$) in the total number WBCs and RBCs) after 33th dpv with significant decrease after 47th dpv. The differential cell counts were different (Fig. 2), the percentage of lymphocytes was significantly increased after 7th dpv and 20th dpv ($P < 0.001$) with a sharp increase after 33th ($P < 0.001$). Neutrophils were fluctuated significantly with time ($P < 0.05$). Monocytes showed non-significant decrease ($P > 0.05$) with a sharp decrease after 47th dpv. In contrast, a significant increase ($P < 0.001$) in the percentage of eosinophil's with a peak after 47th dpv were appeared. Such increased was referred to the natural response of eosinophil's to stress. The percentage of hematocrit showed significant increase ($P < 0.05$) after 33th dpv but, the concentration of hemoglobin was fluctuated significantly ($P < 0.05$) with trend toward increase after 20th dpv, and significant decrease after 33th dpv and 47th dpv (Fig. 3). Both MCH and MCHC showed significant fluctuation with increase after 47th dpv ($P < 0.05$). But non-significant decrease in MCV was showed ($P > 0.05$) (Fig. 3).

For SEA antigen, the total white blood cells were showed in (Table 2, 3, 4, and 5; Figs. 2, 3), which showed significant fluctuations ($P < 0.05$) with time. The red blood cells showed regular trend with significant increase ($P < 0.001$) with time (Fig. 2). The differential cell counts showed in (Fig. 2) demonstrate that significant increase in the percentage of lymphocytes and eosinophil's ($P < 0.001$), with significant decrease in the percentage of neutrophils ($P > 0.05$). The monocytes were fluctuated significantly with a trend of increase ($P < 0.05$). These results were lined with the production of antibodies. The increasing of the red blood cells associated with significant increase in the hematocrit percentage and hemoglobin concentration ($P < 0.001$) which

showed in (Fig. 3). The values of MCV, MCH, MCHC were fluctuated significantly ($P < 0.05$) in MCV and non-significantly in MCH and MCHC ($P > 0.05$) (Fig. 3).

The worm antigen (SWAP) showed the weakest effect on the antibodies production according to ELISA results. This effect appeared on the changes of different blood parameters as a response of worm antigen. The total white blood cells (Fig. 2) showed a significant increase ($P < 0.05$) after 20th dpv, with a trend of significant decrease after 33th dpv and 47th dpv. In contrast the red blood cells increased significantly with time (Fig. 2). The percentage of lymphocytes and eosinophil's were significantly increased ($P < 0.05$) after 47th dpv (Fig. 2). However, the monocytes and neutrophils were significantly fluctuation ($P < 0.05$) with the time with a trend of decrease (Fig. 2). This decrease may be demonstrating the weak effect of worm antigen. The significant increase in number of RBCs caused subsequent significantly increase in the HCT (%) ($P < 0.05$) (Fig. 3). The concentration of hemoglobin was increased significantly ($P < 0.05$) with time (Fig. 3). MCV, MCH and MCHC showed a weak fluctuation with time (Fig. 3). MCV showed a significant increase ($P < 0.05$) after 20th dpv and 33th dpv with significant decrease ($P > 0.05$) after 47th dpv. MCH showed a significant increase ($P < 0.05$) with time which associated with increased hemoglobin concentration. MCHC showed a significant increase with time ($P < 0.05$). The Adjuvant group showed irregular differences in all blood parameters and this difference was associated with a dose and time of injection.

3.2. Lymphocyte blast- transformation rate:

Cells were identified as "blasts" when they were increased in size and exhibited weak chromatin, large nuclei and more voluminous cytoplasm were displayed (Fig. 1).

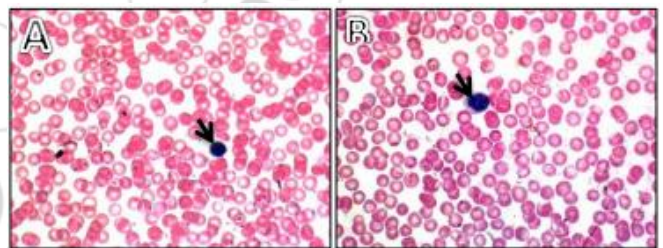


Figure 1: The difference between normal lymphocyte (A) and blast lymphocyte (B)

The blastogenesis rate varied between 10.3 to 44 % in the different groups. The smears showed considerably increased lymphocytes with altered morphology and deeply stained. The result of lymphocyte blastogenesis demonstrated wide variations which correspondence with the antibody titers and haematological results. These results assured the antigenicity of different antigens which were used. As shown in Table (1), the blast cells appeared in the control and vaccinated groups. A maximum percentage of blast cells were seen in CAP vaccinated group after 33 dpv which significantly decreased after 47 dpv. In SEA and SWAP vaccinated groups showed a significant increase of blast cells with time which peaked at 47 dpv for each. The adjuvant group was fluctuated with significant increase in 20 and 47 dpv as a temporary effect.

Table 1: The rate of lymphocytes blastogenesis in the different vaccinated groups

Group Days	7 dpv	20 dpv	33 dpv	47 dpv
Control	18.7 ± 0.57 A(a) (18 – 19)	18.7 ± 0.57 A(a) (18 – 19)	18.7 ± 0.57 A(a) (18 – 19)	18.7 ± 0.57 A(a) (18 – 19)
CAP- vaccinated	10.3 ± 0.57 A(b) (10 – 11)	23.7 ± 0.57 B(b) (23 – 24)	35.3 ± 1.5 C(b) (34 – 37)	20.7 ± 0.57 D(a) (20 – 21)
SEA- vaccinated	14.7 ± 1.5 A(c) (13 – 16)	31 ± 1.0 B(c) (30 – 32)	28.7 ± 0.57 C(c) (28 – 29)	44 ± 1.0 D(b) (43 – 45)
SWAP vaccinated	18.7 ± 1.5 A(a) (17 – 20)	20.3 ± 0.57 A(a) (20 – 21)	35.7 ± 1.5 B(b) (34 – 37)	44 ± 3.4 C(b) (40 – 46)
ADJ- vaccinated	20.3 ± 1.5 A(a) (19 – 22)	30 ± 1.7 B(c) (28 – 31)	22.7 ± 2.5 A(d) (20 – 25)	29.3 ± 0.57 B(c) (29 – 30)

*CAP, cercarial antigen prepared; SEA, soluble egg antigen; SWAP, soluble worm antigen prepared. ** dpv: days post vaccination. ***The different capital letters showing the significant between different periods. The different small letters showing the significant between the control and vaccinated groups at 0.05 levels.

4. Discussion

Extensive studies of both humoral and cellular immune responses to cope with the complexity of the schistosomes life cycle were with the limited success [23]. In this paper, we explore the effect of different types of *Schistosoma* antigens on different blood parameters and the lymphocytic transformed rate of uninfected individual. Three types of *Schistosoma* antigens CAP, SEA and SWAP were studied separately for determining the max titer of antibodies production of each of them and the corresponding changes in the different blood parameters. The previous studies focused on the effect of Schistosomiasis on the different blood parameters [24, 25, 26, 27]. While no haematological studies corresponded to Schistosomal antigens were found. So, one of the major observations in this study was the concord between the antibody titer and the changes in the different blood parameters. Our results were showed a harmonization with ELISA titers which indicated with the peak increase of lymphocytic cells in CAP antigens after 33 days post vaccination, and after 47 days post vaccination for SEA and SWAP vaccinated groups. On the other hand, the neutrophils were decreased in all antigens with time which parallel with high increased in eosinophils percentage. These results were agreement with Swatz et al., [28] which demonstrated that the eosinophils should provide a measure of host defense against endemic infections. It had an important role in immune responses of infected individuals with Schistosomiasis [25]. These results were performed from numerous experiments in vivo [29, 30, 31]. The important role of eosinophils was performed by maintaining the Th2 response to infection via secretion of endogenous IL-4 [32, 33]. Golan et al [27] suggested that the human red blood cells were adhered and lysed by *Schistosomula* of *Schistosomamansoni*. So, the raise of RBCs count will support the immune system in infected individuals. The Schistosomal antigens were showed a significant increase of

RBCs count in SEA and SWAP antigens with time, while CAP antigen had a weak effect on the increasing of RBCs. This increasing was discussed in Schistosomiasis patients by [24].

The total white blood cells did not influenced by neither *Schistosomamansoni* infection in both sexes, nor the density of parasitemia [24]. In our study, the WBCs count showed irregular pattern in the three antigens, but generally were showed a significant increase compared to control group. The hemoglobin concentration was associated with the density of *Schistosomamansoni* infection [24]. In vaccinated groups, an increase of haemoglobin concentration with time was evident. This effect was similar to the percentage of haematocrit, which showed no differences of both sexes, and reversibly affected with the degree of parasitemia [24]. The previous studies were evident that MCH, MCV and MCHC were decreased under infection [34, 24]. Under vaccination, these parameters were showed irregular fluctuations in the three types of antigens.

The increased of lymphocytes in vaccinated groups were accompanied with increase of blastogenesis rate. This rate was studied in several researches under different diseases [13, 14, 35, 36]. These researches were demonstrated a decrease of lymphocyte transformation with infection. In contrast in our material the process of vaccination were associated with an increased lymphocyte transformation with 33 days post vaccination with CAP antigen, and 47 days post vaccination with SEA and SWAP antigens. This increase had a significant relation with antibody production as response of each antigen. Mussatti et al [13] demonstrated that antibodies to soluble antigens seem to correlate with the presence of serum factors, particularly circulating immune complexes. Under the chronic infection of *Schistosomamansoni*, patients exhibited a high specific proliferative response to worm antigen with high lymphoproliferative responses [15]. Contrary to that were investigated by Ottenssen et al., [37] with low proliferative rate as a response of *Schistosoma* worm antigen.

In conclusions, each of Schistosomal antigens were associated with a variable changes in the different blood parameters changes. Studying of these changes in the different blood parameters is a very important tool for understanding the defense and cellular immune mechanism of each antigen against natural infection.

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Table 2: Blood parameters after 7th days post vaccination (mean ± SD)

Parameter	CONTROL	CAP-VACCINATED	SEA-VACCINATED	SWAP-VACCINATED	ADJ-VACCINATED
WBCs (10 ³ cell/mm ³)	6.2 ± 1.0 A (6.9 - 5.1)	9.2 ± 0.9 B (10.2 - 8.6)	3.8 ± 0.4 C (4.1 - 3.4)	4.5 ± 0.8 C (5.2 - 3.8)	4.8 ± 0.5 C (5.2 - 4.3)
LYMP (%)	58.2 ± 2.7 A (60.6 - 55.2)	32.9 ± 0.2 B (44.8 - 41.3)	26.0 ± 1.0 C (27.0 - 25.1)	26.8 ± 0.3 C (27.1 - 26.5)	33.2 ± 1.4 B (34.8 - 32.4)
MONO (%)	8.4 ± 0.5 A (8.9 - 7.9)	9.2 ± 1.5 A (10.9 - 8.2)	12.7 ± 0.3 B (13.0 - 12.4)	7.8 ± 2.5 A (9.9 - 5.9)	6.6 ± 0.5 A (7.1 - 6.1)
NEUT (%)	28.1 ± 1.3 A (29.4 - 26.9)	53.8 ± 3.6 B (57.4 - 50.2)	61.3 ± 0.7 CD (61.9 - 60.6)	65.3 ± 2.3 D (67.1 - 63.3)	59.5 ± 4.4 C (64.5 - 56.5)
EOSIN (%)	1.3 ± 0.3 A (1.5 - 1.0)	0.2 ± 0.2 B (0.3 - 0.0)	0.0 ± 0.0 B (0.0 - 0.0)	0.2 ± 0.1 B (0.3 - 0.0)	0.4 ± 0.5 B (1.0 - 0.0)
HGB (g/dl)	10.0 ± 0.8 A (10.8 - 9.3)	9.1 ± 0.5 B (9.5 - 8.6)	5.1 ± 0.5 C (5.6 - 4.6)	8.5 ± 0.3 BD (8.7 - 8.2)	7.8 ± 0.6 D (8.3 - 7.2)
HCT (%)	26.3 ± 0.6 A (26.8 - 25.6)	27.5 ± 0.9 B (28.5 - 26.9)	15.7 ± 0.3 C (15.9 - 15.4)	24.6 ± 0.6 D (25.1 - 24.0)	22.4 ± 0.4 E (22.8 - 22.0)
RBCs (10 ⁶ /mm ³)	5.2 ± 0.2 A (5.3 - 5.0)	6.1 ± 0.7 B (6.9 - 5.7)	3.7 ± 0.0 C (3.7 - 3.7)	6.2 ± 0.0 B (6.3 - 6.2)	5.2 ± 0.5 A (5.8 - 4.9)
MCV (U ³)	48.7 ± 0.9 A (49.8 - 48.2)	44.6 ± 0.3 B (44.8 - 44.3)	42.1 ± 0.6 C (42.6 - 41.5)	38.9 ± 0.7 D (39.5 - 38.3)	44.5 ± 1.0 B (45.5 - 43.6)
MCH (UUG)	14.4 ± 0.6 AB (14.9 - 13.8)	14.8 ± 0.7 BD (15.4 - 14.1)	13.9 ± 0.4 AC (14.2 - 13.5)	13.2 ± 0.1 C (13.2 - 13.1)	15.5 ± 0.5 D (16.1 - 15.2)
MCHC (%)	31.9 ± 0.8 A (32.6 - 31.1)	33.1 ± 0.1 B (33.2 - 33.0)	33.1 ± 0.3 B (33.3 - 32.8)	34.3 ± 0.3 C (34.6 - 34.0)	35.0 ± 0.1 D (35.0 - 34.9)

Table 3: Blood parameters after 20th days post vaccination (mean ± SD)

Parameter	CONTROL	CAP-VACCINATED	SEA-VACCINATED	SWAP-VACCINATED	ADJ-VACCINATED
WBCs (10 ³ cell/mm ³)	6.0 ± 0.8 A (6.9 - 5.1)	10.7 ± 0.4 B (11.0 - 10.2)	8.9 ± 0.4 C (9.2 - 8.5)	14.2 ± 1.8 D (15.8 - 12.3)	16.5 ± 1.1 E (17.5 - 15.3)
LYMP (%)	52.4 ± 2.8 A (55.6 - 49.8)	43.0 ± 1.8 B (59.8 - 49.2)	32.9 ± 1.7 C (34.8 - 31.7)	42.3 ± 2.0 D (44.1 - 40.2)	25.5 ± 4.6 C (29.6 - 20.6)
MONO (%)	8.0 ± 0.8 AB (8.9 - 6.8)	9.1 ± 1.3 B (10.0 - 7.7)	6.9 ± 0.8 A (7.6 - 6.1)	8.4 ± 0.6 B (8.9 - 7.7)	8.5 ± 0.1 B (8.6 - 8.5)
NEUT (%)	34.8 ± 3.6 A (38.1 - 29.4)	44.2 ± 5.0 B (49.9 - 40.5)	58.1 ± 3.3 C (60.9 - 54.5)	46.0 ± 0.8 B (46.6 - 45.1)	54.1 ± 3.1 C (57.6 - 52.3)
EOSIN (%)	1.2 ± 0.2 A (1.5 - 1.0)	2.6 ± 0.6 BD (3.2 - 2.0)	2.1 ± 0.6 BC (2.6 - 1.5)	3.2 ± 0.5 D (3.8 - 2.8)	1.6 ± 0.4 AC (2.0 - 1.3)
HGB (g/dl)	10.3 ± 0.7 AB (10.8 - 9.3)	11.0 ± 0.8 B (11.9 - 10.3)	9.3 ± 0.2 AC (9.4 - 9.0)	6.7 ± 0.6 D (7.3 - 6.2)	9.2 ± 0.8 C (9.7 - 8.3)
HCT (%)	28.1 ± 0.6 A (28.8 - 27.5)	31.6 ± 0.3 B (31.9 - 31.3)	26.8 ± 1.2 AC (28.1 - 25.9)	18.7 ± 1.0 D (19.4 - 17.6)	26.4 ± 1.2 C (27.3 - 25.0)
RBCs (10 ⁶ /mm ³)	5.5 ± 0.5 AC (6.3 - 5.0)	7.0 ± 0.5 B (7.5 - 6.5)	6.2 ± 0.6 BC (6.9 - 5.7)	4.6 ± 0.5 A (5.1 - 4.2)	6.5 ± 0.6 B (7.3 - 6.1)
MCV (U ³)	49.8 ± 0.4 A (50.2 - 49.2)	45.1 ± 0.4 B (45.5 - 44.7)	43.8 ± 0.6 C (44.4 - 43.4)	41.8 ± 0.3 D (42.0 - 41.5)	39.6 ± 0.4 E (39.9 - 39.2)

MCH (UUG)	14.9 ± 0.8 AC (15.7 – 13.8)	16.1 ± 0.3 B (16.3 – 15.8)	15.2 ± 0.2 BC (15.4 – 15.0)	14.9 ± 0.3 AC (15.2 – 14.7)	13.8 ± 0.9 A (14.9 – 13.2)
MCHC (%)	31.5 ± 0.8 A (32.6 – 30.6)	35.0 ± 0.7 B (35.5 – 34.2)	34.7 ± 0.9 B (35.3 – 33.3)	35.7 ± 0.5 B (36.0 – 35.1)	34.9 ± 1.3 B (36.0 – 33.5)

*The difference of capital letters showing the significant between the control and vaccinated groups at 0.05 levels. CAP, cercarial antigen prepared; SEA, soluble egg antigen; SWAP, soluble warm antigen prepared.

Table 4: blood parameters after 33th days post vaccination (mean ± SD)

Parameter	CONTROL	CAP- VACCINATED	SEA- VACCINATED	SWAP- VACCINATED	ADJ- VACCINATED
WBCs (10³ cell/mm³)	5.8 ± 1.0 A (6.9 – 4.4)	11.3 ± 1.0 B (12.0 – 10.2)	5.4 ± 0.2 A (5.6 – 5.2)	8.4 ± 0.5 C (8.9 – 7.9)	4.6 ± 1.9 A (5.8 – 2.5)
LYMP (%)	52.4 ± 2.8 A (55.6 – 49.8)	52.0 ± 5.4 A (33.1 – 32.6)	42.8 ± 1.5 B (44.3 – 41.3)	32.6 ± 0.5 C (33.1 – 32.1)	42.9 ± 0.9 B (43.9 – 42.3)
MONO (%)	8.0 ± 0.8 AB (8.9 – 6.8)	8.6 ± 0.2 AB (8.8 – 8.4)	9.0 ± 0.5 B (9.5 – 8.5)	10.6 ± 0.5 C (11.1 – 10.1)	7.3 ± 1.3 A (8.8 – 6.5)
NEUT (%)	34.8 ± 3.6 A (38.1 – 29.4)	52.1 ± 0.5 B (52.6 – 51.6)	42.5 ± 1.5 C (44.0 – 41.0)	50.4 ± 1.5 B (51.9 – 48.9)	49.8 ± 2.3 B (51.5 – 47.2)
EOSIN (%)	1.4 ± 0.6 A (2.5 – 1.0)	5.4 ± 0.1 B (5.5 – 5.3)	4.7 ± 0.5 BC (5.2 – 4.2)	4.4 ± 0.5 C (4.9 – 3.9)	3.3 ± 0.7 D (3.9 – 2.6)
HGB (g/dl)	10.3 ± 0.7 A (10.8 – 9.3)	10.6 ± 0.2 A (10.7 – 10.4)	10.9 ± 0.5 A (11.4 – 10.4)	10.7 ± 0.5 A (11.2 – 10.2)	10.6 ± 0.6 A (11.3 – 10.1)
HCT (%)	28.1 ± 0.6 A (28.8 – 27.5)	31.6 ± 0.1 A (31.6 – 31.6)	30.5 ± 0.5 A (31.0 – 30.0)	31.7 ± 0.5 A (32.2 – 31.2)	29.1 ± 9.8 A (39.1 – 19.5)
RBCs (10⁶/mm³)	5.5 ± 0.5 A (6.3 – 5.0)	7.1 ± 0.1 BC (7.1 – 7.1)	7.5 ± 0.1 C (7.6 – 7.4)	7.2 ± 0.2 BC (7.3 – 7.0)	6.9 ± 0.4 B (7.2 – 6.5)
MCV (U³)	49.8 ± 0.4 A (50.2 – 49.2)	43.8 ± 0.4 B (44.2 – 43.4)	38.7 ± 0.8 C (39.4 – 37.9)	42.4 ± 0.3 D (42.7 – 42.1)	41.5 ± 1.0 D (42.6 – 40.8)
MCH (UUG)	14.9 ± 0.8 A (15.7 – 13.8)	14.8 ± 0.2 A (15.0 – 16.4)	13.4 ± 0.3 B (13.7 – 13.1)	14.8 ± 0.5 A (15.3 – 14.3)	15.2 ± 1.3 A (16.6 – 14.1)
MCHC (%)	31.5 ± 0.8 A (32.6 – 30.6)	33.6 ± 0.3 B (33.9 – 33.2)	34.1 ± 0.8 B (34.8 – 33.3)	35.5 ± 0.4 C (35.9 – 35.1)	36.6 ± 0.9 C (37.6 – 36.0)

Table 5: Blood parameters after 47th days post vaccination (mean ± SD)

Parameter	CONTROL	CAP- VACCINATED	SEA- VACCINATED	SWAP- VACCINATED	ADJ- VACCINATED
WBCs (10³ cell/mm³)	5.8 ± 1.0 A (6.9 – 4.4)	6.3 ± 0.6 AB (6.8 – 5.8)	7.6 ± 1.2 C (8.6 – 6.6)	7.4 ± 0.3 BC (7.8 – 1.0)	5.8 ± 0.6 A (6.3 – 5.3)
LYMP (%)	52.4 ± 2.8 A (55.6 – 49.8)	39.7 ± 0.9 B (40.5 – 38.9)	57.3 ± 1.7 C (58.8 – 55.8)	44.9 ± 0.7 D (45.5 – 44.3)	37.3 ± 1.4 B (38.5 – 36.0)
MONO (%)	8.0 ± 0.8 A (8.9 – 6.8)	6.9 ± 0.5 A (7.3 – 6.5)	11.2 ± 0.9 B (11.9 – 10.4)	9.8 ± 0.8 C (10.5 – 9.1)	9.2 ± 0.9 C (10.0 – 8.4)
NEUT (%)	34.8 ± 3.6 AB (38.1 – 29.4)	43.0 ± 2.8 B (45.4 – 40.6)	33.7 ± 4.3 A (37.4 – 30.0)	34.6 ± 0.5 A (35.0 – 34.2)	33.7 ± 0.7 A (34.3 – 33.1)
EOSIN (%)	1.4 ± 0.6 A (2.5 – 1.0)	14.1 ± 1.0 B (15.0 – 13.2)	10.2 ± 0.3 C (10.5 – 9.9)	11.7 ± 0.8 D (12.4 – 11.0)	6.4 ± 0.3 E (6.7 – 6.0)
HGB (g/dl)	10.3 ± 0.7 A (10.8 – 9.3)	8.9 ± 0.1 B (9.0 – 8.8)	13.8 ± 0.2 C (14.2 – 13.3)	12.1 ± 0.7 D (12.7 – 11.0)	12.9 ± 0.5 D (13.3 – 12.5)
HCT (%)	28.1 ± 0.6 A (28.8 – 27.5)	23.7 ± 0.9 B (24.5 – 22.9)	37.9 ± 0.2 C (38.1 – 37.7)	32.5 ± 1.7 D (34.0 – 31.0)	35.3 ± 0.1 E (35.3 – 35.2)
RBCs (10⁶/mm³)	5.5 ± 0.5 A (6.3 – 5.0)	5.9 ± 0.6 A (6.5 – 5.4)	9.2 ± 0.7 B (9.7 – 8.3)	8.1 ± 0.5 C (8.5 – 7.6)	8.6 ± 0.4 BC (9.0 – 8.3)
MCV (U³)	49.8 ± 0.4 A (50.2 – 49.2)	44.2 ± 1.6 B (45.6 – 42.8)	49.2 ± 1.5 A (50.7 – 47.7)	40.4 ± 0.6 C (40.9 – 39.8)	40.8 ± 0.6 C (41.5 – 40.0)
MCH (UUG)	14.9 ± 0.8 AB (15.7 – 13.8)	17.3 ± 1.0 B (18.1 – 16.4)	17.2 ± 2.7 AB (19.5 – 14.8)	15.1 ± 0.2 AB (15.2 – 14.9)	14.9 ± 1.2 A (15.9 – 13.9)
MCHC (%)	31.5 ± 0.8 A (32.6 – 30.6)	36.0 ± 0.1 B (36.0 – 35.9)	32.0 ± 2.3 A (34.0 – 30.0)	37.4 ± 0.3 B (37.8 – 37.0)	36.7 ± 0.7 B (37.6 – 36.0)

*The difference of capital letters showing the significant between the control and vaccinated groups at 0.05 levels. CAP, cercarial antigen prepared; SEA, soluble egg antigen; SWAP, soluble warm antigen prepared.

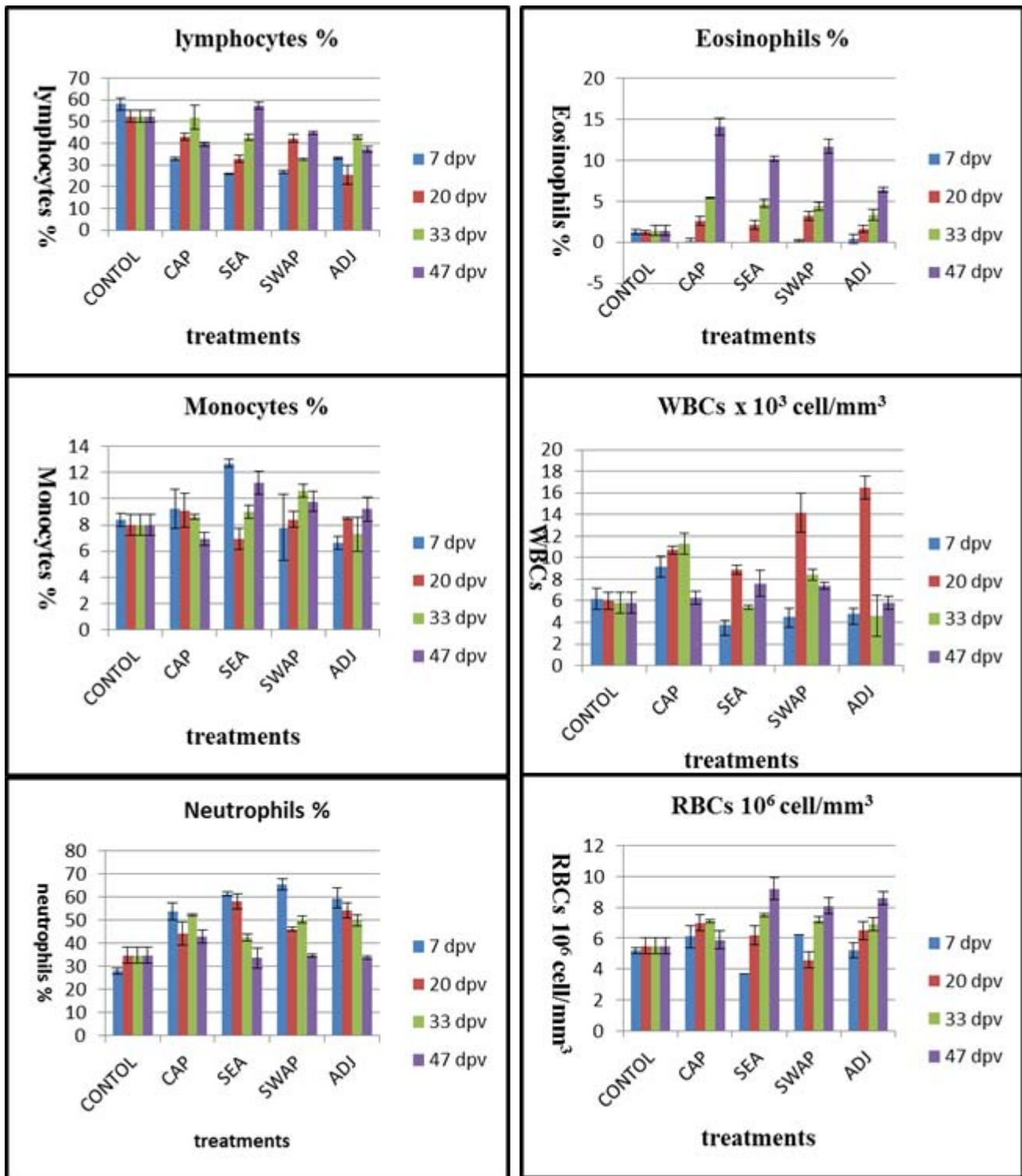


Figure 2: The cellular effect of the Schistosomal antigens on the differential percentage of white blood cells with time of vaccination.

*CAP, cercarial antigen prepared; SEA, soluble egg antigen; SWAP, soluble warm antigen prepared. ** dpv: days post vaccination

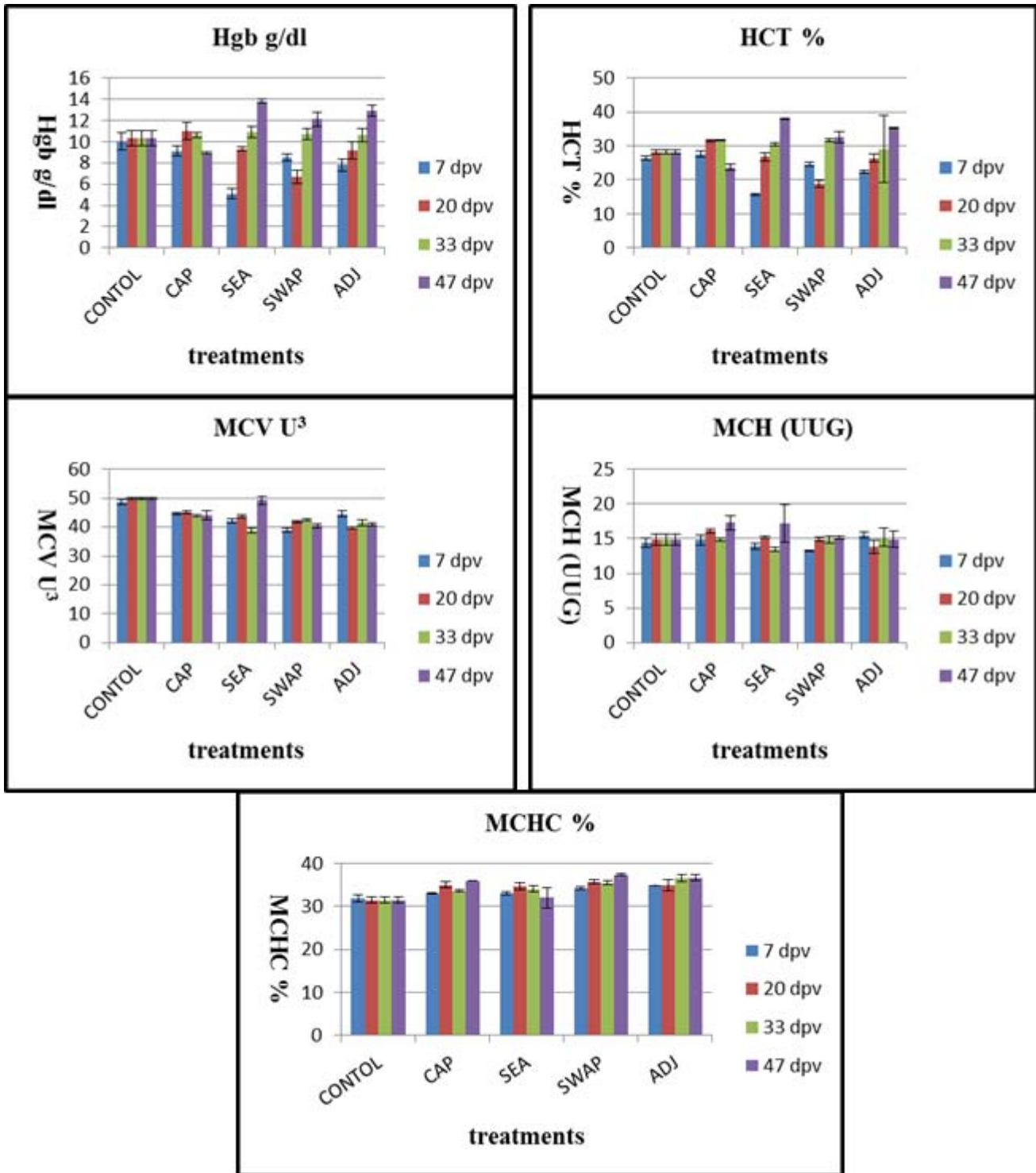


Figure 3: The cellular effect of the Schistosomal antigens on the blood parameters with time of vaccination.

*CAP, cercarial antigen prepared; SEA, soluble egg antigen; SWAP, soluble warm antigen prepared. ** dpv: days post vaccination