Serological And Haematological Responses To Experimental Fascioliasis And Treatment

Nour El Din H. Sherif1, Ismail M. Shalaby1, Maha F. Soliman1, Mohamed G. Hassan2
1Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.
2Parasitology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

Abstract:
This study was established to evaluate and monitor both the humoral and haematological responses to Fascioliasis and its treatment in rabbit model. Rabbits were orally inoculated with viable *F. gigantica* metacercariae and then divided into four groups. Infected rabbits were orally administered a single dose of Triclabendazole (TCBZ) (10 mg kg\(^{-1}\)) at week 4, 8 or 12 post-infection (pi), respectively. Antibody (Ab) response against infection was monitored using enzyme linked immunosorbant assay (ELISA). Total red blood cell counts (Rbcs), haemoglobin content (Hb) and total and differential white blood cells (Wbcs) were also determined. Infected rabbits were found to produce Ab against excretory-secretory products (ESP) of adult flukes two weeks pi where ELISA enabled the early diagnosis of infection. Ab level reached to the peak at week 10 pi. In TCBZ-treated groups, the early Ab responses prior to treatments were similar to response of infected-untreated rabbits. But, after different treatment regimens, Ab levels showed a significant decreases that were depended mainly on both time and hence efficacy of the treatment. In addition, significant reductions in both Rbcs and Hb values accompanied with mild anemia were found among infected group at week 12 &10 pi, respectively. TCBZ-treatment prevented the development of anaemia. Eosinophil numbers significantly increased starting from week 2 pi and peaking at week 4 or 6 pi in all groups. Hence, both eosinophilia and anaemia might be characteristic aspects for experimental fascioliasis.

Key words: Fascioliasis, Triclabendazole, enzyme linked immunosorbant assay, haematology

Introduction
Fascioliasis is a well-known parasitic disease because of its veterinary importance and the great losses it causes in livestock production (Mas-Coma & Bargues 1997). Fascioliasis is also now an important human parasitic disease, with estimated ranging from 2.4 to 17 million people infected. (Mas-Coma *et al.*, 1999). The number of cases of human fascioliasis reported in Egypt has increased drastically during the past years (Curtale *et al.*, 2003).

At present, it is essential that methods to control the disease involve a chemotherapeutic strategy based on epidemiological knowledge. It is therefore necessary to develop methods for early diagnosis and to assay drugs which are effective against developmental stage of *Fasciola* (Martinez, *et al.*, 1997). In this regards, large numbers of fasciolicides are available and used successfully allover the world. According to WHO (1998), Triclabendazole (TCBZ), a benzimidazole compound, has recently been registered in Egypt for the treatment of human fascioliasis. TCBZ has been used in veterinary practice for fascioliasis since 1983. However, time was not too far to recognized that TCBZ resistant liver flukes generated in buffaloes would be transmitted to other ruminant species as well as human (Sanyal, 1998).

Many literatures have been cited about the role of enzyme-linked immunosorbent assay (ELISA) in diagnostic purpose for fascioliasis (Levine *et al.*, 1980, Cornelissen *et al.*, 1992; Guobadia & Fagbemi, 1996; Martinez *et al.*, 1996). On the other hand, little is known about the various mechanisms of immunomodulation that occur either during fascioliasis or
during disease treatment compared to informations available for other trematode infections like *Schistosoma* (Poitou *et al.*, 1992; Martinez *et al.*, 1997).

A broad spectrum of mammalian species especially herbivorous animals may serve as experimental hosts for the common liver flukes, *Fasciola sp*. Establishment of *F. gigantica* infection in the laboratory animals has been previously reported in mice (Yoshihara & Suzuki, 1990), in rat (Yoshioka, 1991), in guinea pig (EL-Shaieb, 1995) and in rabbit (Abou-Basha *et al.*, 1983; Desoky, 1985, Gupta & Yadav, 1995; EL-Sayed, 1997; Abdel-Rahman *et al.*, 1997; El-Sayed, 1997; Helal *et al.*, 2000; Abdel-Rahman & Abdel-Megeed, 2004). In this regard, El-Bahy (1997) suggested that the best condition for production of mature *F. gigantica* worm in rabbit depends mainly on the dose of metacercariae and the general health conditions of the rabbit.

The present work was established for supporting the studies required for diagnosis and control of fascioliasis. The humoral and haematological responses to experimental fascioliasis alone and combined with the chemotherapy (TCBZ) in rabbit model were monitored.

**Material and methods**

**Animals & Experimental Infection**

Twenty five rabbits (approximately, 2 - 2.5 kg) were used for the establishment of *F. gigantica* infection. The rabbits were kept in clean metal cages and fed on balanced ration and water *ad-libitum*. The rabbits were healthy and free from parasites and infectious diseases as indicated by the absence of clinical signs and by performing blood analysis as well as the daily faecal examination for 10 days using the simple sedimentation technique (Faust, *et al.*, 1939). Twenty rabbits were orally inoculated with 25 ± 5 viable *F. gigantica* metacercariae (age: 10-15 days) per animal; the viability of metacercariae were determined by the refractile appearance of the excretory granules (Boray, 1969) and the movement of juveniles within the cysts. The remaining five rabbits were served as control group (A).

**Treatment Regimens**

Infected rabbits were randomly allocated in four groups of five animals each (groups B, C, D & E). Group B remained infected and did not receive treatment. At weeks 4, 8 and 12 post infection (pi), TCBZ was administered to groups C, D and E, respectively. TCBZ (FASINEX: CIBA-GEIGY, Switzerland) was orally administered as a single therapeutic dose of 10 mg kg⁻¹ in distilled water. The dose was calculated according to Paget & Barnes (1964).

**Blood Sampling:**

Blood samples were taken from the eye of all rabbits by using orbital sinus technique (Sanford, 1954) at weeks 0, 2, 4, 6, 8, 10, 12, 14, and 16 pi. Blood samples were collected into tubes containing the anticoagulant (Ethylene Diamine Tetra – Acetate, EDTA) at the rate of 2 mg/ml of blood (Hawk *et al.*, 1965) for haematological analysis and also into tubes without anticoagulant for preparation of sera and serological analysis. Obtained sera were stored at -20°C till used in antibodies assay.

**F. gigantica Excretory – Secretory Product (ESP) (Antigen Preparation):**

ESP antigen was prepared according to Rivert Marrero *et al.* (1988). Flukes were collected from cattle bile ducts and washed several times in 0.01 M phosphate buffered saline (PBS), pH 7.4 and then incubated in PBS at 37°C for 3 hrs; one worm/ 5 ml in PBS. The flukes were removed and the supernatant fluid (PBS + ESP) was centrifuged at (12, 000 rpm) for 1 hr at 4°C. The supernatant was separated and designated as ESP antigen. The protein content was determined by Biomeriux kit (laboratory reagent and instrument, France) according to Lowry *et al.* (1951). The antigen was aliquoted and stored at −70°C until used.

**Enzyme Linked Immunosorbent Assay (ELISA):**

Specific antibodies against *F. gigantica* ESP antigen were determined during infection and treatment using ELISA (Oldham, 1983). Microtiter plate (Nunc) was coated with 100 μl ESP antigen (5 μg
protein /ml) in coating buffer (0.05 M carbonate buffer, pH 9.6) and incubated overnight at 4°C. The plates were washed in PBS – Tween (PBS—T) – 20 (0.05 %) then 200 µl/ well of blocking buffer, 0.1% bovine serum albumin, (BSA) was added and incubated for 1 hr at 37°C. 100 µl of serum of all experimental groups diluted at 1:100 in PBS were added and incubated for 1hr at room temperature. After washing, 100 µl / well of the conjugate, Horseradish peroxidase conjugated goat anti rabbit IgG (whole molecule) (Sigma Immunoc-chemicals) was added 1:2000 dilution in PBS–T 0.05% (were added). The plates were incubated for 1hr at 37°C. After washing, substrate, Ortho–phenylenediamidine (Sigma Immunochemicals) at 100 µl / well and incubated for 5 minutes at room temperature. The reaction was stopped by adding 100 µl / well of 1 M H₂SO₄. The optical density (OD) was read using spectrophotometer (Titertek – multiscan, McC photometer {Flow Lab, Mclean, Virginia} at 492 nm.

Haematological parameters:

The total red blood cells (Rbcs) and white blood cells (Wbcs) were counted per µl of blood by Neubauer double haemocytometer according to Miller & Seward (1971). The blood haemoglobin (Hb) and differential Wbcs counts were determined according to Jain (1986).

Flukes Recovery and Drug Efficacy:

At week 16 pi, all infected and treated rabbits were sacrificed and dissected. Flukes were recovered according to the method of Kendall et al. (1967) where livers and gall bladders were examined carefully for flukes; all recovered flukes were collected and counted. The anthelmintic efficacy of each drug was expressed as percentage reduction of the number of flukes in the treated groups when compared with the untreated control group, according to the following formula: (Mean number flukes in control group) – (Mean number of flukes in treated group) / (Mean number flukes in control group) X100.

Histological examination

Specimen of infected liver were removed out from scarified rabbits, fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm thickness and stained with H&E.

Statistical analysis

All data were conducted with the software packages Microsoft SPSS version 11.0, for statistical evaluation. Data were presented as means ± SE. A student’s t-test and one way analysis of variance (ANOVA) were used.

Results

Monitoring of Ab response in all the experimental groups using ELISA is shown in Fig (1). In infected-untreated rabbits, group (B), Ab level began to rise above the levels of non-infected group (A) 2-week pi (p < 0.01). After that there was a significant increase in Ab level, reaching to the peak at week 10 pi. Ab level showed a slight decrease at the end of the experiment (week 16 pi).

In TCBZ-treated groups (C–E), the early antibody response before treatment was similar to that of group B. The peak of Ab levels of group C, treated at week 4 pi, was recorded at week 6 pi and after that the level remained nearly constant till week 12 pi followed by significant decrease at weeks 16 pi compared to level of group B (p < 0.001). At this time Ab level was still above level of non-infected rabbits (p < 0.001). Ab level reached to the peak at weeks 10 & 12 pi in groups D & E, respectively. Ab levels showed a significant decrease 6 & 2 weeks post-treatment in groups D and E, respectively. At week 16, Ab level of groups D and E was significantly lower than level of group B (p < 0.001), but still exceed levels of pre-infection (P < 0.001). At week 16 pi, a significant difference in the Ab levels was found between groups D and E (P <0.05).

Monitoring the changes in Rbcs, Hb in all groups throughout the time of experiment is shown in figures (2 & 3). Remarkable decrease in both Rbcs and Hb values (mild anaemia) of infected rabbits (group B) were found at weeks 12 & 10 pi, respectively, when compared to non-infected group A (p < 0.01). This decrease extended to week 16 pi (p <0.001). In TCBZ-treated groups (C & D), Rbcs & Hb values were within the normal range. While...
in group E, a significant decrease below normal range was found starting from week 14 and 12 pi in Rbcs & Hb values, respectively ($p < 0.01; p < 0.001$).

Concerning the total Wbcs count, it was within the normal levels in all experimental groups. But, only the eosinophils showed a significant increase 2-weeks pi, peaked at weeks 4 or 6 pi and followed by significant decrease in their number; although it was still above the normal levels (Fig. 4). No changes were recorded in the number of neutrophils, basophils, monocytes and lymphocytes; they were all around the normal values.

Regarding to TCBZ efficacy, our results showed that TCBZ was highly efficient in eliminating the flukes and its efficacy was dependent on time of administration. The efficacy was 93.75 %, 100% & 100% for groups treated at weeks 4, 8 & 12 pi, respectively. Figure (1) showed *Fasciola gigantica* worm resided in the bile ducts of an infected rabbit.

**Table 1: Antibody Levels (Measured By ELISA) against *F. gigantica* ESP antigen during experimental fascioliasis and TCBZ-Treatment.** Absorbance at 492 nm. Results are given as mean ± SE. A: Group A: Non-infected; B: Infected-untreated; C: Infected-treated- 8 weeks pi; D: Infected-treated- 4 weeks pi; E: Infected-treated- 12 weeks pi.

<table>
<thead>
<tr>
<th>Weeks pi</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.13 ± 0.03</td>
<td>0.13 ± 0.04</td>
<td>0.4 ± 0.03</td>
<td>0.153 ± 0.02</td>
<td>0.131 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>0.16 ± 0.03</td>
<td>0.217 ± 0.01</td>
<td>0.238 ± 0.02</td>
<td>0.226 ± 0.02</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>0.14 ± 0.03</td>
<td>0.33 ± 0.2</td>
<td>0.33 ± 0.02</td>
<td>0.32 ± 0.03</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>0.13 ± 0.04</td>
<td>0.364 ± 0.03</td>
<td>0.364 ± 0.03</td>
<td>0.39 ± 0.02</td>
<td>0.372 ± 0.02</td>
</tr>
<tr>
<td>8</td>
<td>0.14 ± 0.04</td>
<td>0.387 ± 0.02</td>
<td>0.39 ± 0.02</td>
<td>0.421 ± 0.02</td>
<td>0.366 ± 0.02</td>
</tr>
<tr>
<td>10</td>
<td>0.153 ± 0.02</td>
<td>0.485 ± 0.04</td>
<td>0.47 ± 0.03</td>
<td>0.49 ± 0.02</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>12</td>
<td>0.13 ± 0.04</td>
<td>0.463 ± 0.03</td>
<td>0.484 ± 0.04</td>
<td>0.433 ± 0.05</td>
<td>0.033 ± 0.03</td>
</tr>
<tr>
<td>14</td>
<td>0.152 ± 0.02</td>
<td>0.412 ± 0.2</td>
<td>0.392 ± 0.02</td>
<td>0.254 ± 0.01</td>
<td>0.273 ± 0.03</td>
</tr>
<tr>
<td>16</td>
<td>0.14 ± 0.03</td>
<td>0.385 ± 0.02</td>
<td>0.272 ± 0.04</td>
<td>0.215 ± 0.02</td>
<td>0.266 ± 0.05</td>
</tr>
</tbody>
</table>

**Table 2: Changes in RBCs (x10^6/mm^3) count during Fascioliasis and TCBZ- Treatment.**

Results are given as mean ± SE. A: Group A: Non-infected; B: Infected-untreated; C: Infected-treated- 8 weeks pi; D: Infected-treated- 4 weeks pi; E: Infected-treated- 12 weeks pi.
Serological And Haematological Responses To………..

Table 3: Changes in HB (g %) content during experimental Fascioliasis and TCBZ-Treatment.

<table>
<thead>
<tr>
<th>Weeks pi</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.78±0.8</td>
<td>11±0.4</td>
<td>10.65±0.5</td>
<td>11.38±1.0</td>
<td>10.9±0.1</td>
</tr>
<tr>
<td>2</td>
<td>10.75±0.4</td>
<td>11.28±0.9</td>
<td>10.55±0.6</td>
<td>10.63±1.3</td>
<td>10.8±0.5</td>
</tr>
<tr>
<td>4</td>
<td>10.75±0.6</td>
<td>10.95±0.5</td>
<td>10.68±0.7</td>
<td>11.1±0.9</td>
<td>10.93±0.9</td>
</tr>
<tr>
<td>6</td>
<td>10.35±0.7</td>
<td>10.75±0.6</td>
<td>10.98±0.9</td>
<td>10.83±1.3</td>
<td>10.78±0.9</td>
</tr>
<tr>
<td>8</td>
<td>9.88±0.4</td>
<td>10.1±0.6</td>
<td>10.53±0.4</td>
<td>10.2±0.7</td>
<td>10.95±1.0</td>
</tr>
<tr>
<td>10</td>
<td>9.38±0.2</td>
<td>9.43±0.4</td>
<td>10.3±0.4</td>
<td>9.28±0.3</td>
<td>10.6±0.7</td>
</tr>
<tr>
<td>12</td>
<td>8.25±0.24</td>
<td>8.9±1.2</td>
<td>9.95±0.4</td>
<td>8.48±0.4</td>
<td>10.85±0.5</td>
</tr>
<tr>
<td>14</td>
<td>8.45±0.2</td>
<td>9.35±0.2</td>
<td>10.1±0.4</td>
<td>8.4±0.1</td>
<td>10.28±0.5</td>
</tr>
<tr>
<td>16</td>
<td>8.88±0.3</td>
<td>9.38±0.2</td>
<td>10.4±0.8</td>
<td>8.15±0.3</td>
<td>11.1±0.83</td>
</tr>
</tbody>
</table>

Results are given as mean ± SE. A: Group A: Non-infected; B: Infected-untreated; C: Infected-treated- 8 weeks pi; D: Infected-treated- 4 weeks pi; E: Infected-treated- 12 weeks pi.

Table 4: Changes in number of eosinophils during experimental Fascioliasis and TCBZ-Treatment.

<table>
<thead>
<tr>
<th>Weeks pi</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3±2.0</td>
<td>3.1±1.2</td>
<td>2.3±1.3</td>
<td>2.5±1.2</td>
<td>3.5±1.7</td>
</tr>
<tr>
<td>2</td>
<td>15.5±5.1</td>
<td>9.5±3.1</td>
<td>20±8</td>
<td>17±6.1</td>
<td>5±2.0</td>
</tr>
<tr>
<td>4</td>
<td>35.5±4.7</td>
<td>25±7.8</td>
<td>31±10.6</td>
<td>33±7.7</td>
<td>2.1±1.4</td>
</tr>
<tr>
<td>6</td>
<td>29±7.8</td>
<td>19±6.5</td>
<td>35±7.3</td>
<td>24±8</td>
<td>1.8±1.2</td>
</tr>
<tr>
<td>8</td>
<td>11.5±4.4</td>
<td>18.5±7.5</td>
<td>14.5±5.2</td>
<td>10±3.9</td>
<td>4.8±2.5</td>
</tr>
<tr>
<td>10</td>
<td>7.5±1.6</td>
<td>8±1.7</td>
<td>6.5±3</td>
<td>8±4.8</td>
<td>3.8±1.1</td>
</tr>
<tr>
<td>12</td>
<td>11.3±3.0</td>
<td>10.5±3.3</td>
<td>6.5±6.5</td>
<td>6±2.1</td>
<td>5.5±2.2</td>
</tr>
<tr>
<td>14</td>
<td>8.2±1.8</td>
<td>6.5±3.5</td>
<td>10.5±5.6</td>
<td>13.5±6.4</td>
<td>3.7±1.8</td>
</tr>
<tr>
<td>16</td>
<td>12±2.4</td>
<td>9±3.7</td>
<td>7.3±1.7</td>
<td>9.5±1.1</td>
<td>2.5±1.9</td>
</tr>
</tbody>
</table>

Results are given as mean ± SE. A: Group A: Non-infected; B: Infected-untreated; C: Infected-treated- 8 weeks pi; D: Infected-treated- 4 weeks pi; E: Infected-treated- 12 weeks pi.

Fig 1 (A): Liver section of rabbit experimentally infected with F. gigantica showing the worm resided in the bile ducts. F: fluke; bd: bile duct; hc: hepatic cells; t tegument of worm (x100). (B): enlarged liver section (x200).
Discussion

Using direct ELISA method, Ab against ESP of adult *F. gigantica* was found early, two weeks pi, in sera of the experimentally infected rabbits where ELISA enabled the early diagnosis. This method, which has been used in other infections, showed satisfactory responses when employed in this study. Antibody level reached peak at week 10 pi. This antibody response was similar to that described for the primary infection with *Fasciola sp.*; in rat (Levine, et al., 1980), sheep (Cornelissen et al., 1992; Chauvin et al., 1995), cattle (Boulard et al., 1995) in goats (Martinez et al., 1996) and rabbit (El Bahy, 1997). On the other hand, the detection of antibodies by first week pi was recorded in rat (Yoshioka, 1991; Poitou, et al., 1992) and in sheep (Guodadia & Fagbemi 1996) and by week 3 in rabbit (Santiago et al., 1986) and rat (Keegan & Trudgett, 1992). Helal et al. (2001) found that Ab were detected in the sera of rabbits infected with *F. gigantica* at 6 week pi against adult and metacercarial antigen and at weeks 9-10 pi against ESP using precipitating test. Hence, these findings may confirm the advantage of using direct ELISA methods.

Antibody peak observed in this study at week 10 pi indicated that there is a different Ab response at this late stage of infection compared to the early low response. In this regard, Poitou et al. (1992) pointed out that the antibody response is basically directed against antigen determinants of high molecular weight, present both in the juvenile tegument and in ESP structure in the adult. However, Hillyer (1979) found that ESP appeared more antigenic than tegument antigen using Eliza. This finding has been also supported by Chauvin et al. (1995), who suggested that the production of antibody appearing at the late stage of infection are related specifically to the sequential release of ESP by adult flukes in bile ducts.

In TCBZ-treated groups, the early antibody responses were similar to those of infected-untreated rabbits followed by significant decrease after treatment that was depended on both the time and efficacy of treatment. These findings are similar to those of Levine et al. (1980) in rat, Hanna, et al. (1982) in sheep, Fetterer, et al. (1985) in cattle and Martinez et al. (1997) in goats. Fetterer, et al. (1985) suggested that the reduced Ab response after treatment must be expected if drug treatment kills flukes while they are immature when antigen used in ELISA was prepared from adult flukes. In addition, it has been reported that when the early treatment failed to achieve total elimination of parasites, the post-peak decrease was marked after several weeks post-treatment, while, in goats in which treatment totally eliminated the parasite burden, Ab levels fell back very rapidly to negative values (Martinez et al., 1997). In contrary, our results showed that although there was a high significant drop in Ab levels at weeks 6 & 2 post treatment in groups D & E, respectively, where there was total elimination of flukes, the levels did not reach to negative values at all. The reason of this variation in serological response between our study and the study of Martinez et al., (1997) may be related to the difference in immune response of the experimental animals used which could modify the mode of action of TCBZ treatment. For instance, TCBZ which was able to eliminate the ESP of flukes in goats may not be able to have the same effect in rabbit. However, these points need a further investigation.

The current study showed that experimental fascioliasis caused a mild anaemia starting from weeks 10-12 pi in untreated-infected rabbits. Since at weeks 10 or 12, usually the late immature or mature worms are found in bile ducts, the anemia might be directly related to the presence of worms in the ducts (Hawkins, 1984 and Martinez et al., 1997). In contrary, Haroun, et al. (1989) found anemic process in *F. gigantica*-infected goats at the start of infection that coincided with the migration of immature forms through the hepatic parenchyma. This study showed that TCBZ-treatment resulted in absence of anaemic processes when
treatment was applied early in groups C & D with 94% & 100% fluke eradication rates, respectively. Only in group E, treated at week 12 pi, anaemia was found but the improvement may be expected in the followings weeks. However, many other factors might play a role in the anemic process during fascioliasis like size of the fluke burden (Hawkins, 1984) and species of animals (Waweru et al., 1999).

Eosinophilia, involved in hypersensitivity mechanisms and phagocytosis, is a feature of helminthes infestation, including Fasciola (Poitou et al., 1993 and Chauvin et al., 1995). Accordingly, a monophasic eosinophilia with one peak at week 4 or 6 was found in all infected and treated groups. Chauvin et al. (1995) described a biphasic eosinophilia with two peaks recorded from week 3-5 pi followed by drop and second increase from week 9-11 pi in F. hepatica infected sheep. In contrary, Martinez et al. (1997) reported that the leukocytes number were highly variable and no significant difference were detected between the non-infected, infected and treated goats experimentally infected with F. hepatica. However, we may suggest that both eosinophilia and anaemia might be characteristic aspects for experimental fascioliasis.

References


وهو ما يثبت أهمية اختبار الإليزا في التشخيص المبكر للعدوى. وصل مستوى الأجسام المناعية إلى الذروة في الأسبوع العاشر بعد الأصابة. أما بالنسبة إلى المجموعات المعالجة فقد كانت الاستجابات المناعية الأولية قبل العلاج مشابهة لاستجابات الأرانب المصابة وغير المعالجة. ولكن بعد المعالجة فإن الاستجابات المناعية أظهرت اختلافات معنوية و التي اعتمدت على عناصر الوقت والتالي كفاءة المعالجة. وفيما يخص بالقياسات الدموية، فقد أوضحت الدراسة أن أعداد كرات الدم الحمراء وكذلك محتوى الهيموجلوبين قد تأثرت بالسلب (فقر دم طفيف) في الحيوانات المصابة بداية من الأسبوع 10 و 12 بعد الإصابة، على التوالي. هذا وقد منع العلاج المبكر هذه التغييرات الدموية. وتشير الدراسة إلى أنه كان هناك إضافة زيادة ملحوظة في عدد الكرات المحبة للحمض الذي وبلغت الذروة في الأسبوع 4 أو 6 في كل المجموعات. وبالتالي يمكن القول بأن كل من فقر الدم و زيادة أعداد الكرات المحبة للحمض قد يكونان من السمات المميزة للإصابة بالفاشياولا عملياً.