The Egyptian Journal of Hospital Medicine Vol., 18 : 124 - 132 March 2005
I.S.S.N: 12084
1687 - 2002

Serological And Haematological Responses To Experimental Fascioliasis And Treatment

Nour El Din H. Sherif[1], Ismail M. Shalaby[1], Maha F. Soliman[1], Mohamed G. Hassan[2]

[1]Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.
[2]Parasitology 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 eosniophilia 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-Bahy, 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 haema-tological analysis and also into tubes without anticoagulant for preparation of sera and serological analysis. Obtained sera were stored at -20^{o}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 ^{o}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^{o}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 ^{o}C until used.

Enzyme Linked Immunosorbant 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-T 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-hemicals) 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–phenylenediam-idine (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 H_2SO_4. 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.

Weeks pi	Group A	Group B	Group C	Group D	Group E
0	0.13 ± 0.03	0.13 ± 0.04	0.4 ± 0.03	0.153 ± 0.02	0.131± 0.03
2	0.16 ± 0.03	0.217 ± 0.01	0.238 ± 0.02	0.226 ± 0.02	0.23 ± 0.03
4	0.14 ± 0.03	0.33 ± 0.2	0.33 ± 0.02	0.32 ± 0.03	0.32 ± 0.03
6	0.13 ± 0.04	0.364 ± 0.03	0.364 ± 0.03	0.39 ± 0.02	0.372 ± 0.02
8	0.14 ± 0.04	0.387 ± 0.02	0.39 ± 0.02	0.421 ± 0.02	0.366 ± 0.02
10	0.153 ± 0.02	0.485 ± 0.04	0.47 ± 0.03	0.49 ± 0.02	0.36 ± 0.04
12	0.13 ± 0.04	0.463 ± 0.03	0.484 ± 0.04	0.433 ± 0.05	0.0.33 ± 0.03
14	0.152 ± 0.02	0.412 ± 0.2	0.392 ± 0.02	0.254 ± 0.01	0.273 ± 0.03
16	0.14 ± 0.03	0.385 ± 0.02	0.272 ± 0.04	0.215 ± 0.02	0.266 ± 0.05

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.

Weeks pi	Group A	Group B	Group C	Group D	Group E
0	4.53±0.2	4.33±0.2	4.6±0.9	4.48±0.1	4.97±0.3
2	4.35±0.2	4.33±0.3	4.45±0.2	4.45±0.5	4.47±0.3
4	4.35±0.3	4.45±0.1	4.45±0.5	4.4±0.2	4.7±0.1
6	4.25±0.3	4.23±0.3	4.2±0.2	4.1±0.4	4.25±0.2
8	4.05±0.3	4.08±0.1	4.35±0.3	4.48±0.2	4.38±0.3
10	3.73±0.2	3.75±0.2	4.33±0.3	4.18±0.3	4.38±0.2
12	3.6±0.2	3.72±0.2	3.88±0.3	3.55±0.1	4.4.1±0.3
14	3.48±0.2	3.83±0.3	3.9±0.2	3.4±0.3	4.55±0.2
16	3.43±0.2	3.75±0.1	4.13±0.5	3.15±0.3	4.13±0.5

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.

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

Weeks pi	Group A	Group B	Group C	Group D	Group E
0	10.78±0.8	11±0.4	10.65±0.5	11.38±1.0	10.9±0.1
2	10.75±0.4	11.28±0.9	10.55±0.6	10.63±1.3	10.8±0.5
4	10.75±0.6	10.95±0.5	10.68±0.7	11.1±0.9	10.93±0.9
6	10.35±0.7	10.73±0.6	10.98±0.9	10.83±1.3	10.78±0.9
8	9.88±0.4	10.1±0.6	10.53±0.4	10.2±0.7	10.95±1.0
10	9.38±0.2	9.43±0.4	10.3±0.4	9.28±0.3	10.6±0.7
12	8.25±0.24	8.9±0.2	9.95±0.4	8.48±0.4	10.85±0.5
14	8.45±0.2	9.35±0.2	10.1±0.4	8.4±0.1	10.28±0.5
16	8.88±0.3	9.38±0.2	10.4±0.8	8.15±0.3	11.1±0.83

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.

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

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.

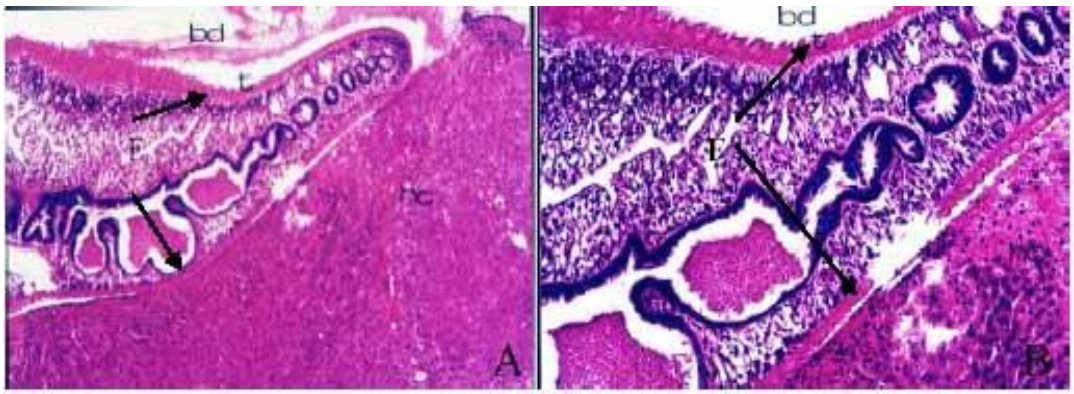

Fig 1 (A): Liver section of rabbit experimentally infected with *F. gigantica* showing the worm resided in the bile ducts. F: fluke; bd: bile duct; hc: hepatic cells; t tegument of worm (x100). (B): enlarged liver section (x200).

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 hypersen-sitivity 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 eosniophilia and anaemia might be characteristic aspects for experimental fascioliasis.

References

1. **Abdel-Rahman, E.H. and Abdel-Megeed, K. N. (2004):** *Fasciola gigantica*: immuni-zation of rabbits with proteins isolated from coproantigen. J Egypt Soc Parasitol., 34(2): 631-642.
2. **Abdel-Rahman, M.S., El~Bahy MM. and EI-Bahy N.M. (1997):** Testing the parasit-icidal efficacy of fental (nitazoxanide). Alex. J. Vet Science, 13(4): 447-458
3. **Abou-Basha, L. M.; Elmagdoub, A. A.; Ebeid, S. A.; Toukhy,M.A.; Michael, A. I.; Hosny, K. M.; El-Zoghby, S. M.; Faraq, H. F. and El-Sawy, M.F. (1983):** Effect of bithionol on B-glucuronidase in serum and liver of rabbits infected with *Fasciola gigantica*. J. Egypt. Soc. Parasitol., 13(1): 231-238.
4. **Boray, J. C. (1969):** Experimental fasciol-iasis in Australia. Adv. Parasitol., 7: 95-210.
5. **Boulard, C.; Carreras, F. and Van Gool, F. (1995):** Evaluation of nitroxynil and closantel activity using ELISA and egg counts against *Fasciola hepatica* in experimentally and naturally infected cattle. Vet. Res., 26:249-255.
6. **Chauvin, A.; Bouvet, G. and Boulard, C. (1995):** Humoral and cellular immune responses to *Fasciola hepatica* experimental primary and secondary infection in sheep. Int. J. Parasitol., 25(10):1227-1241.
7. **Cornelissen, J.B.; De Leeuw, W. A. and Van der Heijden, P. j. (1992):** Comparison of an indirect haemagglutination assay and an ELISA for diagnosing *Fasciola hepatica* in experimentally and naturally infected sheep. Vet. Quarterly, 14 (4): 152-156.
8. **Curtale F, Abd El-Wahab Hassanein Y, El Wakeel A., Mas-Coma S. and Montresore A. (2003):** Distribution of human fascioliasis by age and gender among rural population in the Nile Delta, Egypt. J Trop Pediatr., 49(5):264-8.
9. **Desoky, E.A (1985):** Identification of antibodies against *Fasciola gigantica* in natural and experimental hosts using antigen from the development stages in snails. Ph.D. Thesis, Department of Parasitology, Faculty of Veterinary Medicine, Zagazig University.
10. **El-Bahy, M. M. (1997):** Rabbit as a model for experimental fasciol1asis in Egypt. Alex. J. Vet. Sc., (13): 5
11. **El-Sayed, M. H. (1997):** Comparative studies on the effect of bithionol, praziq-uantel and triclabendazole in rabbit's fascio-liasis. 1. Parasitological studies .J. Egypt. Soc. Parasitol., 27(1):131-42.
12. **El-Shaieb, A. F., (1995):** Experimental pathologic studies on *Fasciola gigantica* and *Trichinella spiralis* in guinea pigs. Ph.D. Thesis, Department of Parasitology, Faculty of Veterinary Medicine, Zagazig University.
13. **Faust, E. C.; Sawitz, W.; Tobile, J.; Pres, C. and Lincicim, D. R. (1939):** Comp-arative efficiency of

various techniques for the diagnosis of protozoa and helminthes in faeces. J. Parasitol., 25:241-262.

14. **Fetterer, R. H.; Rew, R. S.; Gasbarre, L. C. and Ostlind, D. A. (1985):** Prophylactic efficacy of clorsulon against *Fasciola hepaticca* in calves and sheep. Vet. Parasitol., 18: 21-27
15. **Guobadia, E. E. and Fagbemi, B. O. (1996):** Detection of circulating *Fasciola gigantica* antigen in experimental and natural infections of sheep with fascioliasis. Vet. Parasitol., 65 (1-2): 29-39.
16. **Gupta, S. C. and Yadav, S. C. (1995):** Antibody response of rabbit to enhanced doses of *Fasciola gigantica* experimental infection. J. Vet. Parasitol. : 73-77.
17. **Hanna, R. E.; Hughes, D. L. and Taylor, S. M. (1982):** *Fasciola hepatica*: antibody levels in sheep serum before and after treatment with antihelmintic. Res. Vet. Sci., 51(1): 328-332.
18. **Haroun, E. M.; El-Sanhouri, A. A. and Gameel, A. A. (1989):** Response of goats to repeated infections with *Fasciola gigantica*. Vet. Parasitol., 30(4): 287-296.
19. **Hawk, P. B., Oser, B. L. and Summerson, W. (1965):** Hawk's physiological chemistry. 14th London. J. and Aohurchill. pp. 33.
20. **Hawkins, C. D. (1984):** The use of haemoglobin, packed cell volume and serum sorbitol dehydrogenase as indicators of the development of fascioliasis in sheep. Vet Parasitol., 15:125-133.
21. **Helal, I. B., Moustafa, H. E. and Nabila M. M. (2000):** Host-parasite interaction in rabbit fascioliasis. I. C. B. S., 1(2) :595-612.
22. **Hillyer, G. V.(1980):** Isolation of Fasciola hepatica tegument antigen**s.** J. Clinc. Microbiol., 12(5): 695-699.
23. **Jain, N. C. (1986):** Veterinary haematology 4th, Lea and Febiger, Philadelphia, USA
24. **Keegan, P. S. and Trudgett, A. (1992):** *Fasciola hepatica* in the rat: immune respo-nses associated with the development of resistance to infection. Parasite Immunol., 14: 657-669.
25. **Kendall, S. B.; Hebert, N. J.; Parfitt, J. W. and Peirce, M. A. (1967):** Resistance to reinfection with *Fasciola hepatica* in rabbits. Exp. Parasitol., 20: 242-247.
26. **Levine, D. M.; Hillyer, G. V. and Flores, S. I. (1980):** Comparison of counterelectro-phoresis, the enzyme-linked immunosorbent assay, and Kato fecal examination for the diagnosis of Fascioliasis in infected mice and rabbits. Am. J. Am. J. Trop. Med. Hyg., 29(4):602-608.
27. **Lowry, O. H., Rosenbrough, N. J., farr, A. L. and Randall, R. J. (1951):** Protein measurement with folin-phenol reagent. J. Biol. Chem., 193: 265-275.
28. **Martinez, A.: Martinez-Cruz, M. S.; Martinez, F. J.; Gutierrez, P. N. and Hernandez, S. (1996):** Detection of antibodies to *Fasciola hepatica* excretory–secretory antigens in experimentally infected goats by enzyme linked immunosorbent assay. Vet. Parasitol., 62:247-252.
29. **Martinez A., Martinez-Moreno F. J., Acosta I.,Gutierrez P. N., Becerra C. and Hernandez S.(1997):** Humoral and cellular immune responses to experimental *Fasciola hepatica* infections in goats. Parasitol. Res., 83(7): 680-686.
30. **Mas-Coma S. and Bargues M.D, (1997):** Human liver flukes: a review. Res Rev Parasitol., *57:* 145–218
31. **Mas-Coma M. S., Esteban J. G. and Bargues M. D. (1999**): Epidemiology of human fascioliasis: a review and proposed new classification. Bull World Health Organ; 77(4):340-346.
32. **Miller, S. E. and Seward, J. M. (1971):** Textbook of clinical pathology. 8th Ed, The willians and Wilking Company, Baltimore. pp. 22.
33. **Oldham, G. (1983):** Antibodies o *Fasciola hepatica* antigens during experimental infections in cattle measured by ELISA. Vet. Parasitol., 13: 151-158.
34. **Paget, G. E. and Barnes, J. M. (1964):** Evaluation of drug activities. Vol.1, Academic Press.

35. **Poitou, I.; Baeza, E. and Boulard, C. (1992):** Humoral and cellular immune responses in rats during a primary infestation with *Fasciola hepatica*. Vet. Parasitol., 45(1-2):59-71.
36. **Poitou, I.; Baeza, E. and Boulard, C. (1993):** Kinetic responses of parasite-specific antibody isotypes, blood leucocytes pattern and lymphocyte subsets in rats during primary infestation with *Fasciola hepatica*. Vet. Parastiol., 49: 179-190.
37. **Rivert Marrero, C. A.; Santiago, N. and Hiyller, G. V. (1988):** Evaluation oh immuneodiagnostic antigens in the excretory-secretory products of Fasciola hepatica. J. Parasitol., 74(4):646-652.
38. **Sanford, H. S. (1954):** Method for obtaining venous blood from the orbital sinus of the rat or mouse. Science, **119**: 100.
39. **Santiago, N.; Hillyer, G. V.; Garcia-Rosa, M. and Morales, M. H. (1986):** Identif-ication of functional *Fasciola hepatica* antigens in experimental infections in rabb-its. Am. J. Trop. Med.Hyg., 35(1):135-140.
40. **Sanyal, P.K. (1998):** Pharmacokinetics behavior of Triclabendazole in domestic ru-minants following single and divided dose adinistration. J. Vet. Parasitol., 12(2): 89-93.
41. **Waweru, J. G.; Kanyari, P. W.; Mwangi, D. M.; Ngatia, T. A. and Nansen, P. (1999):** Comparative parasitological and haematological changes in two breeds of sheep infected with *Fasciola gigantica*. Trop.Anim.Health.Prod., 31(6):363-372.
42. **WHO (1998):** Triclabendazole and fascioliasis - a new drug to combat an age-old disease. Fact sheet no. 191. Geneva.
43. **Yoshihara, S. and Suzuki, K. (1990):** Antigens and antibodies in the ascitic fluid of mice infected with *Fasciola gigantica*. Vet. Parasitol., 35: 175-178.
44. **Yoshioka, Y. (1991):** Immunodiagnostic and pathological studies on experimental fascioliasis in rats. Jpn. Nara. Med. Ass., 42:587-603.

استجابات سيرولوجية و دموية على الإصابة بالفاشيولا و علاجها

نور الدين شريف -إسماعيل شلبى – مها سليمان - محمد جمال حسن

قسم علم الحيوان- كلية العلوم – جامعة قناة السويس- الإسماعيلية – جمهورية مصر العربية [1]

قسم الطفيليات – كلية الطب البيطرى- جامعة قناة السويس- الإسماعيلية – جمهورية مصرالعربية [2]

فى هذه الدراست تمت مراقبة وتقييم الإستجابات المناعية و الدموية للإصابة بالفاشيولا معملياً و علاجها. تمت إصابة الأرانب بميتاسركاريات فاشيولا جايجانتيكا عن طريق الفم وبعد ذلك تشعّبت الأرانب المصابة إلى أربع مجموعات. تم علاج 3 مجموعات من الأرانب المصابة بجرعة واحدة عن طريق الفم من عقار تريكلابندازول (10 مج كجم$^{-1}$) فى بداية الأسابيع 4 و 8 أو 12 بعد الإصابة. تم تقييم و مراقبة الإستجابة المناعية ضد الإصابة بالفشيولا باستخدام المواد الإخراجية للدوده البالغة عن طريق اختبار الإليزا. كذلك تم تقييم عدد كرات الدمّ الحمراء، محتوى الهيموجلوبين والعدد الكلى و النوعى لخلايا الدمّ البيضاء خلال فترة الدراسة. أوضح اختبار الإليزا وجود اجسام مناعية فى الأرانب المصابة عند الأسبوع الثانى من العدوى المعملية

وهو ما يثبت أهمية اختبار الإليزا فى التشخيص المبكّر للعدوى. وصل مستوى الأجسام المناعية إلى الذروة في الأسبوع العاشر بعد الأصابة. اما بالنسبة الى المجموعات المعالجة فقد كانت الاستجابات المناعية الأولية قبل العلاج مشابهة لاستجابات الأرانب المصابة و غير المعالجة. ولكن بعد المعالجة فإن الاستجابات المناعية أظهرت اختلافات معنويه و التي إعتمدت على عنصر الوقت و بالتالى كفاءة المعالجة. و فيما يخص بالقياسات الدمويه، فقد أوضحت الدراسة أن أعداد كرات الدم الحمراء و كذلك محتوى الهيموجلوبين قد تأثرت بالسلب (فقر دم طفيف) فى الحيوانات المصابة بداية من الأسبوع 10 و 12 بعد الإصابة، على التوالي. هذا و قد منع العلاج المبكر هذه التغييرات الدموية. و تشير الدراسة الى انه كان هناك ايضا زياده ملحوظه فى اعدد الكرات المحبة للحمض و التى وبلغت الذروة في الأسبوع 4 او 6 في كلّ المجموعات. و بالتالى يمكن القول بان كل من فقر الدم و زيادة اعداد الكرات المحبة للحمض قد يكونان من السمات المميزة للإصابة بالفاشيولا معملياً.