Egypt. J. Agric. Res., 79 (2) , 2001.

EFFECT OF *TRICHINELLA SPIRALIS* INFECTION ON SOME DIAGNOSTIC INDICES IN DOGS

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(Manuscript received 15 April 2000)

Abstract

Ten parasite free dogs were grouped into 2 groups:

Group I, a negative control and Group II, infected with 5000 *T.spiralis* larvae / each dog. *Trichinella spiralis* infected dogs under study became off food, and diarrhoeic by the 2nd up to the 7th day post-infection. Vomiting has been observed in one of the infected dogs. Fever was recorded on the 2nd up to the 21st day post-infection.

The main blood changes were significant decrease in haemoglobin concentration (7th – 21st days P.I.) and mean corpuscular haemoglobin concentration (MCHC) by the 7th day P.I. together with a significant increase in total eosinophil and leukocyte counts (4th – 21st days P.I.).

Serum biochemical alterations were detected on the 7th day up to the 28th day P.I. (end of experiment) depending on the test used. Serum biochemical changes included a significant decrease in total serum proteins, albumin, urea and creatinine concentrations, together with increased serum globulin concentration and serum ALT activity levels.

It could be concluded that, blood and serum diagnostic indices are supplementary tests during vague trichinosis symptoms.

INTRODUCTION

Trichinosis is a cyclozoonotic disease characterized by three successive stages: [1] intestinal, [2] muscle invasion, and [3] convalescence (Beaver & Jung, 1984). The clinical signs which accompany trichinosis of man and animals, are very variable and may simulate those of a variety of other diseases. They include, diarrhoea, fever, retroperitoneal pain, dispnoea, pain of affected muscles, depression and sometimes oedema of the face (Soulsby, 1982, Beaver & Jung, 1984 and Bowman *et al.*, 1991).

Few of the reviewed literatures dealt with the clinical signs, haematological and serum biochemical changes accompanying dog trichinosis. Among them were Bowman, (1991) and Reina, *et al.* (1989), who detected minor signs of gastrointestinal upset, a slight eosinophilia and detectable alterations in plasma protein levels during the course of experimental trichinosis in dogs.

The present report aimed at studying the effect of *T.spiralis* infection on some haematological diagnostic indices.

MATERIALS AND METHODS

Material

1. Animals

Ten mongrel pups, less than two months old were separated from their mothers and kept individually in sufficiently clean-screened bottom-wire cages, on hygienic nutritive diets. Faecal and blood samples were examined parasitologically over two months period to ensure that they are parasite-free. The dogs were grouped into two groups (each of five).

Group I : Negative control.
Group II : *T.spiralis* infected.

2. *Trichinella spiralis* larvae

T.spiralis larvae were obtained from the digested diaphragms of infected pigs according to the method described by Nabih, (1978).

Parasitological Examination

Faecal and blood samples were examined according to the methods described by Allen and Ridley (1970) and Coles (1986).

Infective *T.spiralis* larvae obtained from the diaphragms of infected pigs were collected, maintained and propagated in white rats according to the methods described by Nabih, (1978). Collected *T.spiralis* larvae were suspended in 20 % gelatin saline (37° C). After a thorough mixing, 0.1 ml of the suspension was spread onto a microscope slide and the whole larvae were counted to determine the appropriate infective dose (Mikhail and Tadros, 1973). A minimum of three counts were made to determine the mean count / 0.1 ml of suspension. Dogs of the IInd group received 5000 larvae / each dog via a stomach tube.

Blood samples were collected from all dogs 5 days before the infection procedure and on the 4th , 7th , 15th , 21st and 28th days post-infection (P.I.) of the IInd group. Whole blood samples, were examined for haemoglobin concentration, packed cell volume, total eosinophils and leukocyte counts according to the methods described by

Coles (1986).

Serum samples, were used for the determination of total proteins according to Hoffamann and Richterrich (1970), albumin and globulins (Doumas, *et al.*, 1971), Urea (Patton and Crouch, 1977), creatinine (Henry, 1974) and aminotransferases (ALT and AST) according to Reitman and Frankel, (1957).

Data was analyzed statistically using the Student's t-test, and differences between means were considered significant at p-value less than 0.05.

RESULTS

The observed clinical signs of acute trichinosis among infected dogs, under study, included rise of body temperature by the 2^{nd} up to the 21^{st} day post-infection, dogs became off food and diarrhoeic by the 3rd up to the 7^{th} day post-infection. Vomiting has been observed in one of the infected dogs. Blood and serum changes during acute trichinosis, were presented in Tables 1, 2, 3 and 4.

DISCUSSION

The observed clinical signs of acute trichinosis among infected dogs under study, were similar to the clinical symptoms of acute trichinosis of man and animals recorded by Vasilinin (1983), Beaver and Jung (1984), Kolokl'tsev *et al.* (1987) and Bowman *et al.* (1991).

Data presented in Table 1, showed significant decrease in haemoglobin concentration, among *T.spiralis* infected dogs, between the 7^{th} –21^{st} days post-infection (P.I.) together with a significant decrease in mean corpuscular haemoglobin concentration (MCHC) by the 7^{th} day P.I. The decreased haemoglobin and MCHC in *T.spiralis* infected dogs, under study, are in complete agreement with Curca *et al.* (1996) findings, and could be attributed to the decreased iron absorption through the degenerated intestinal mucosa (Reina *et al.*, 1989) and the lowered liver/iron levels in *T.spiralis* infection, recorded by Theodoropoulos and Greve (1986).

Data presented in Table 2 points to a significant increase in leukocyte and total eosinophil counts of *T.spiralis* infected dogs under study. Eosinophilia together with leukocytosis, observed between the 4^{th} – 21^{st} days P.I., are in general agreement with the findings recorded by Vasilinin (1983), Figallová and Prokopic, (1988), Soffar (1990) and Bowman *et al.* (1991). The observed eosinophilia and leukocytosis, among

Table 1. Haemoglobin, Packed Cell Volume and Mean Corpuscular Haemoglobin Concentration (mean ± S.E.) in Free and *T.spiralis* Infected Dogs.

Time	Haemoglobin (g/dl)		P.C.V. (%)		M.C.H.C. (g/dl)	
	G. I (n = 5)	G. II (n = 5)	G. I (n = 5)	G. II (n = 5)	G. I (n = 5)	G. II (n = 5)
Pre-infection	13.5 ± 0.12	13.6 ± 0.10	41.6 ± 1.40	40.3 ± 0.86	32.7 ± 1.28	33.8 ± 0.95
4th d. P.I.	13.6 ± 0.11	13.4 ± 0.12	41.5 ± 1.05	40.0 ± 0.84	32.8 ± 1.04	33.6 ± 0.99
7th d. P.I.	13.6 ± 0.21	12.1* ± 0.42	42.3 ± 0.63	41.8 ± 1.51	32.2 ± 0.74	28.9* ± 0.91
15th d. P.I.	13.6 ± 0.28	11.6** ± 0.30	42.8 ± 1.65	39.3 ± 0.45	31.1 ± 0.59	29.6 ± 0.74
21st d. P.I.	13.5 ± 0.19	12.8* ± 0.16	43.4 ± 1.63	40.0 ± 0.64	31.2 ± 1.13	31.9 ± 0.51
28th d. P.I.	13.6 ± 0.16	13.8 ± 0.16	42.1 ± 1.48	44.6 ± 0.62	32.6 ± 1.38	31.0 ± 0.64

Table 2. Total Leukocyte and Total Eosinophil Counts (mean ± S.E.) in Free and *T.spiralis* Infected Dogs.

Time	Leukocyte Count (cell/µl)		Eosinophil Count (cell/µl)	
	G. I (n = 5)	G. II (n = 5)	G. I (n = 5)	G. II (n = 5)
Pre-infection	9990 ± 388	10150 ± 575	231 ± 60	199 ± 19
4th d. P.I.	10140 ± 375	12710** ± 727	206 ± 40	1288*** ± 160
7th d. P.I.	10290 ± 350	14880*** ± 1087	241 ± 64	1546*** ± 129
15th d. P.I.	9680 ± 293	11590** ± 532	265 ± 63	1968*** ± 85
21st d. P.I.	10210 ± 270	13770*** ± 978	276 ± 40	768*** ± 33
28th d. P.I.	9690 ± 419	10660 ± 276	250 ± 40	576*** ± 46

Dog eosinophilia > 750 cell/µl blood (Coles, 1986)

* = Significant at P< 0.05 ** = Significant at P< 0.02

*** = Significant at P< 0.05

Table 3. Total Blood Proteins, Albumin and Globulins concentrations (mean ± S.E.) in Free and *T.spiralis* Infected Dogs.

Time	Total Proteins (g/dl)		Albumin (g/dl)		Globulins (g/dl)	
	G. I (n = 5)	G. II (n = 5)	G. I (n = 5)	G. II (n = 5)	G. I (n = 5)	G. II (n = 5)
Pre-infection	6.76 ± 0.23	7.12 ± 0.17	3.70 ± 0.08	3.94 ± 0.10	3.06 ± 0.21	3.18 ± 0.14
4th d. P.I.	7.18 ± 0.22	6.92 ± 0.30	3.92 ± 0.15	3.82 ± 0.12	3.26 ± 0.12	3.10 ± 0.35
7th d. P.I.	7.22 ± 0.15	6.14*** ± 0.08	3.96 ± 0.14	3.76 ± 0.05	3.26 ± 0.26	2.38 ± 0.05
15th d. P.I.	7.44 ± 0.18	7.48 ± 0.07	3.94 ± 0.15	3.46 ± 0.14	3.50 ± 0.32	4.02 ± 0.10
21st d. P.I.	7.30 ± 0.15	7.12 ± 0.22	3.90 ± 0.17	3.20* ± 0.25	3.40 ± 0.19	3.92* ± 0.15
28th d. P.I.	7.14 ± 0.18	7.16 ± 0.24	3.88 ± 0.11	3.28*** ± 0.12	3.26 ± 0.09	3.88** ± 0.12

Table 4. Urea, Creatinine, ALT and AST Serum Concentrations (mean ± S.E.) in Free and *T.spiralis* Infected Dogs.

Tests	Groups n = 5	Time of Sampling					
		Pre-infection	4th d. P.I.	7th d. P.I.	15th d. P.I.	21st d. P.I.	28th d. P.I.
Urea	G. I	19.9 ± 1.8	20.8 ± 1.1	20.1 ± 0.9	23.6 ± 2.5	21.4 ± 2.1	21.1 ± 1.3
(mg/dl)	G.II	18.1 ± 1.8	17.5 ± 1.1	8.7*** ± 1.5	8.6*** ± 0.8	22.8 ± 1.4	24.0 ± 2.5
Creatin.	G. I	0.82 ± 0.06	0.84 ± 0.06	0.80 ± 0.05	0.78 ± 0.04	0.80 ± 0.06	0.75 ± 0.02
(mg/dl)	G.II	0.77 ± 0.01	0.80 ± 0.03	0.64** ± 0.01	0.66* ± 0.01	0.76 ± 0.04	0.74 ± 0.03
ALT	G. I	32.2 ± 2.2	29.2 ± 2.5	32.1 ± 2.6	27.8 ± 1.7	28.9 ± 1.9	32.5 ± 2.9
(U/ml)	G.II	36.9 ± 2.0	41.2* ± 2.8	36.9 ± 1.3	45.8*** ± 4.1	47.2** ± 5.1	36.9 ± 1.
AST	G. I	31.3 ± 2.9	30.9 ± 1.6	30.5 ± 2.2	26.9 ± 2.7	29.6 ± 1.2	29.1 ± 2.8
(U/ml)	G.II	32.3 ± 1.9	30.6 ± 2.4	34.8 ± 1.2	29.6 ± 0.9	40.0 ± 6.1	39.8 ± 4.1

* = Significant at P< 0.05

** = Significant at P< 0.02

*** = Significant at P< 0.01

T.spiralis infected dogs, represent a body reaction to the invasive stages of the parasite within the infected dogs under study.

Table 3 demonstrates a significant decrease in serum total proteins on the 7^{th} day P.I. of the *T.spiralis* infected dogs under study. Significant decrease in serum albumin together with a significant increase in serum globulins was detected between the $21^{st} - 28^{th}$ day P.I.

The detected alterations in serum proteins, among *T.spiralis* infected dogs under study, are in general agreement with the findings recorded by Przyjalkowski and Wolf-Golabeck (1985) and Reina *et al.* (1989). The decreased serum total proteins detected by the 7^{th} day P.I. of the *T.spiralis* infected dogs, could be attributed to the seized food uptake (Coles, 1986) during the intestinal phase of trichinosis. The latter decrease of serum albumin levels ($21^{st} - 28^{th}$ day P.I.) could be a matter of the liver pathological changes, recorded by Kr"stev *et al.* (1986), Reina *et al.* (1989) and El-Nokaly *et al.* (1997) in *T.spiralis* infection. The significantly increased serum globulin concentration by the 21^{st} day post-infection denotes an immunological response of *T.spiralis* infected dogs under study.

Table 4 denotes a significantly decreased serum urea concentration ($7^{th} - 15^{th}$ day P.I.), among *T.spiralis* infected dogs, that could be attributed in one part to the decreased food uptake (Coles, 1986) during the intestinal phase of *T.spiralis* infection, and in the other part to the hepatic pathological changes recorded by Kr"stev *et al.* (1986), Reina *et al.* (1989) and El-Nokaly *et al.* (1997). The significantly decreased serum creatinine concentration ($7^{th} - 14^{th}$ day P.I.) in *T.spiralis* infected dogs could be a sign of increased glomerular filtration as a result of glomerulonephritis recorded by Todarova *et al.* (1992) in *T.spiralis* infected animals. This, in turn, is greatly explained by the fact that "Creatinine is excreted by glomerular filtration, and significant quantities are neither excreted nor reabsorbed by the tubules" (Coles, 1986).

The significantly increased serum ALT levels among *T.spiralis* infected dogs ($4^{th} - 21^{st}$ days P.I.), are indicative of hepatic changes. Such increased serum ALT levels agreed with Baiorinene and Firantene (1989) findings and could be attributed to the hepatic pathological changes in livers of *T.spiralis* infected animals, recorded by Kr"stev *et al.* (1986), Reina *et al.* (1989) and El-Nokaly *et al.* (1997).

Serum AST levels revealed no significant variation at any of the experiment time, between the non-infected control and the *T.spiralis* infected group of dogs under study.

From the obtained results of the present study, it could be concluded that, blood and serum diagnostic indices are supplementary tests during trichinosis symptoms. Decreased haemoglobin serum total proteins, albumin, urea and creatinine concentrations, together with increased total eosinophil and leukocyte counts, serum globulin concentration and serum ALT activity levels, might be indicative of acute trichinosis among feverish dogs with gastrointestinal upset.

REFERENCES

1. Allen, A.V.H. and D.S. Ridley. 1970. Further observations on the formol-ether concentration technique for faecal parasites. J. Clin. Pathol., 23: 545-546.

2. Baiorinene, D.V. and R.K. Firantene. 1989. Changes in the activity of some hepatic enzymes caused by parasitic diseases. Acta Parasit. Lituanica, 23: 83-87.

3. Beaver, P.C. and R.C. Jung. 1984. Clinical Parasitology. 9th Ed. Lea and Febiger, Philadelphia, USA.

4. Bowman, D.D., M.F. Frongillo, K.B. Johnston and R.C. Johnson. 1991. Signs, larval burdens, and serological responses of dogs experimentally infected with *Trichinella spiralis* Owen, 1835. Folia Parasit., 38: 245-253.

5. Coles, E.H. 1986. Veterinary clinical pathology. 4th Ed. W.B. Saunders, Philadelphia and London.

6. Curca, D., G. Olteanu and L.I. Mitrea. 1996. Haematological and biochemical aspects of experimental trichinellosis in pigs. Medicina Vet., 39: 51-61.

7. Doumas, B., W. Watson and H. Biggs. 1971. Albumin standards and measurement of serum albumin with bromcresol green. Clin. Chem. Acta, 31: 87-92.

8. El-Nokaly, A.A., A.A.A. El-Aziz, N.M. Kamal and D.M. Amin. 1997. Morphological and histochemical studies on liver of albino mice infected with *Trichinella spiralis* before and after fluconazole (diflucan) therapy. Egypt. J. Comp. Pathol., Clin. Pathol., 10: 119-132.

9. Figallová, V. and J. Prokopic. 1988. The dynamics of eosinophilia in concurrent infections with *Trichinella spiralis* Owen, 1835 and *Toxascaris leonina* Linstow, 1909. Folia Parasit., 35: 365-369.

10. Henry, R.J. 1974. Clinical Chemistry, Principles and Technics. 2nd Ed. Harper and Row, P. 525.

11. Hoffamann, T.P. and R. Richterrich. 1970. Die El iminerong von Trubungen beider Bestimmung von plasma proteinan mitden Buiret Rasgen. Z. Klin. Chem. U. K. in Biochem, 8: 595.

12. Kolokol'tsev, M.M., A.P. Fedoseev, I.A. Kolokol'tseva and E.A. Zhitnitskaya. 1987. Adaptation of a natural strain of Trichinella to domestic pigs. Meditsin. Parazitol. Parazitar. Bol., 4: 40-41.

13. Kr"stev, L., I. Dimitrova, S. Komandarev and O. Polyakova-Kr"steva. 1986. [Comparative pathology in experimental trichinelliasis in mice infected by *Trichinella spiralis*, *T.pseudospiralis*, *T.nelsoni* and *T.nativa*]. Khelmintol., 22: 25-32.

14. Mikhail, E.G. and M.B. Tadros. 1973. Dipterex in experimental trichinosis. J. Egypt. Soc. Parasit., 3: 69.

15. Nabih, A. 1978. Use of the serodiagnostic methods for the evaluation of the meat inspection procedures for trichinosis in pigs slaughtered in Alexandria slaughterhouse. Thesis, M.P.H. High Inst. Publ. Hlth, Alex. Univ.

16. Patton, C.J. and S.R. Crouch. 1977. Spectrophotometric and investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem., 49: 464-469.

17. Przyjalkowski, Z. and D. Wolf-Golabeck. 1985. Further electrophoretic investigations of the changes in the blood serum protein fractions of germ-free and conventional mice infected with single or mixed *Trichinella spiralis* and *T.pseudospiralis* larvae. Acta Parasit. Polonica, 30 (I / II): 73-85.

18. Reina, D., M. Habela, I. Navarrete and F. Martinez-Gómez. 1989. Hypoglycaemia in experimental canine trichinellosis. Vet. Parasit., 33 (3-4): 289-296.

19. Reitman, S. and S. Frankel. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-68.

20. Soffar, S.A. 1990. Pattern of eosinophilia and muscle larval burdens in experimental trichinellosis. J. Egypt. Soc. Parasit., 20: 841-847.

21. Soulsby, F.J.L. 1982. Helminths, arthropods and protozoa of domesticated animals. 7th Ed. ELBS, Baillers and Tindall, London, U.K.

22. Theodoropoulos, G. and J.H. Greve. 1986. Relationship between blood iron parameters and trichinellosis in swine. Vet. Parasit., 19 (1 /2): 121-126.

23. Todarova, V., S. Bojkov and K. Noeva. 1992. Determination of tissue-bound immune complexes in kidneys of rats with experimental trichinellosis by means of immunoelectron microscopy. Khelmintol., 32: 59-66.

24. Vasilinin, M.G. 1983. Combined use of anthelmintics and antibiotics in the treatment of experimental trichinellosis. Sibirskoe Otdelenie VASKhNIL, 14-17. (Helm. Abst., 55: 560)

أثر الإصابة بالتريكينيللا سبيراليس على بعض القياسات التشخيصية في الكلاب

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أجريت هذه الدراسة على عدد عشرة كلاب عمر ٣ – ٤ أشهر خالية من الأمراض الطفيلية حيث تم تقسيمها إلى مجموعتين (خمسة كلاب بكل مجموعة):

المجموعة الأولى: مجموعة الكنترول الضابطة ، أحتفظ بها خالية من العدوى.

المجموعة الثانية: مجموعة الاختبار ، تم إحداث العدوى بها بعدد خمسة آلاف يرقة من يرقات ديدان التريكينيللا سبيراليس لكل كلب منها. وقد ظهرت أعراض الامتناع عن الطعام ، الإسهال بين أفراد مجموعة الاختبار خلال اليوم الثاني إلى اليوم السابع من تاريخ العدوى. كما تم تسجيل حدوث حالات القيء في كلب واحد فقط من أفراد هذه المجموعة. هذا بينما استمر الارتفاع في درجة حرارة الجسم طوال الحادي والعشرين يوما التالية لإحداث العدوى.

وبدراسة تأثير الإصابة بالتريكينيللا سبيراليس على بعض القياسات التشخيصية لعينات الدم والسيرم أمكن إثبات حدوث انخفاض معنوي حقيقي في تركيز هيموجلوبين الدم مع حدوث ارتفاع معنوي حقيقي في تعداد خلايا الدم البيضاء وخلايا الدم الحمضية خلال الحادي والعشرين يوما التالية للعدوى. كما أثبتت هذه الدراسة حدوث انخفاض معنوي حقيقي في تركيز كل من البروتين الكلي ، الألبومين ، اليوريا ، الكرياتينين مع حدوث ارتفاع معنوي حقيقي في تركيز جلوبيولينات السيرم وإنزيم الألانين أمينو ترانسفيريز نتيجة للإصابة بالتريكينيللا سبيراليس بينما لم يحدث تغير معنوي في تركيز إنزيم الأسبرتات أمينو ترانسفيريز بين الأفراد المصابة.

مما سبق يوصي الباحثون باستخدام القياسات التشخيصية المختلفة لعينات الدم والسيرم كاختبارات مساعدة لتشخيص الإصابة بالتريكينللا سبيراليس لدى الاشتباه في تواجد الإصابة بمرض التريكينوسيس الحاد والذي تتشابه أعراضه بصورة كبيرة مع أعراض التسمم الغذائي.