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Biochemical and Histopathological Changes in Balb/C Mice-As an Experimental Animal Model- Infected with *Taenia saginata* Oncospheres

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Abstract: This study aimed to use BALB/c mice as an animal model to develop *Taenia saginata* oncosphere infection. The biochemical and histopathological changes in this model were estimated. Tumor-like cysts containing meta-cysticerci were obtained at the inoculation sites of mice at the 8th week post-infection which confirmed by using polymerase chain reaction PCR assay. The 1,300 base pair (bp) product was detected for *T. saginata* cyst by PCR. Histpathological examination of infected mice heart revealed myocarditis. Biochemical analysis result showed that there was a significant (P<0.05) increase in serum globulins and marked decrease in A/G ratio in group of mice infested with oncospheres of *T. saginata* compared to non-infested group. However, there was no significant difference in serum total proteins and albumin of both groups of treatment. Serum of creatinine and total cholesterol levels and ALT activity in infested group were markedly (P<0.01) increased compared to control non-infected group. In conclusion, female BALB/c mice can be used as experimental animals for studying the host immune response in vaccine development trails. As well as *T. saginata cysticercosis* caused an alteration in liver and kidney functions.

Key words: *Taenia saginata* · Oncospheres · Cysticercosis · Balb/C Mice · PCR · Serum Biochemistry · Pathology

INTRODUCTION

Taenia saginata is a medically and economically important cestode parasite. Infection with the cysticercus larval stage in cattle causes economic loss in the beef meat industry. The life cycle of human tapeworm, *Taenia saginata* involves humans as definitive host for the tapeworm and cattle as the intermediate host for the larval stage. Cattle become infected after eating *T. saginata* eggs (proglottids) from infected humans. Once cattle are infected, cysticerci develop in the muscle and subsequently become infective to humans after approximately 10 weeks [1, 2]. Eggs that are released before defecation usually become mixed with feces. Eggs may be distributed directly onto the ground. An infected human host is generally asymptomatic, although mild gastrointestinal signs may occur [3].

According to the literature, BALB/c mice are less resistant than Swiss mice to the experimental intra- peritoneal (I/P) infestation with *T. crassiceps cysticerci*. Also these mice have been used as an experimental model for *T. solium* cysticercosis [5]. Cysticercosis of *T. solium, T. saginata* and *T. saginata asiatica* has also been studied in severe combined immunodeficient (SCID) mice [6, 7].

T. saginata cysticercosis caused an alteration in liver and kidney functions in infested cattle [8]. Moreover, *Taenia saginata asiatica* cysticerci damaged host (pig) liver tissues leads to the metabolic disorder of lipid, glycogen, protein and changes in enzyme metabolism [9]. Heavy infestation by *T. saginata* cysticercosis may cause myocarditis and heart failure in cattle [10].

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Therefore, the objective of the study was to investigate upon the susceptibility of female BALB/c mice-as animal model- inoculated with hatched oncospheres of *T. saginata* for infection and its development into mature cysticerci to be used for further investigations in the experimental vaccination. Also, to determine its effect on serum biochemical parameters related to liver and kidney functions. The mature cysticerci formation in infected mice was confirmed using PCR assay.

MATERIALS AND METHODS

This study was carried out according to guidelines for animal experimentation and approved by the Institutional Animal Care and Use Committee, National Research Centre Animal Care Unit, Dokki, Giza, Egypt.

Parasite: Adult worms of *T. saginata* were obtained from infested patients with taeniasis in Assuit hospital, Assuit governorate, Egypt.

Egg Collection and Hatching: The eggs of *T. saginata* were collected from the gravid proglottids of each worm, stored at 4°C. *In vitro* hatched oncospheres were carried out according to method of sodium hypochlorite (0.5% in normal saline) technique [11, 12]. The viability of the oncospheres was assessed by a microscopic examination using 0.4% Trypan blue solution (dissolved in distilled water) at the ratio of the Trypan blue solution to the oncospheres sample was 1:10. After one minute, the viable eggs didn't change in colour and the dead ones were stained blue under a microscope [12].

Animals: Twenty-five mice BALB/c strain weighing 20 to 25 g of 6-8 weeks old were purchased from the Animal House, Theodor Bilharz Research Institute, Giza, Egypt. The mice were housed in a well-ventilated animal room under standardized conditions (20±3°C; relative humidity 50±5% and 12 hours light/dark cycle). All nutrients including water were supplied *ad libitum* to meet the requirements of the NRC [13]. Mice were acclimatized for 7 days before the start of the experiment.

Experimental Infection: Twenty-five female Balb/c mice were divided into 2 groups, the first group (n=10) was kept as control (non-infected) and the other infected group (n=15) was inoculated intra-peritoneal with 5,000 oncospheres/mice. *Post-mortem* examinations were done at the 8^{th}, 12^{th}, 14^{th} and 20^{th} weeks post infection. Blood samples were collected for estimation of biochemical at the end of experimental [11].

PCR Assay: Diagnosis of cysticerci infection is confirmed by DNA analysis. DNA was extracted from cysts according to the instruction of DNA extraction kit protocol by by QIAamp Kits (Qiagen, Valencia, Calif, USA). Primer designation based on the sequence of ribosomal gene as shown in Table (1) according to Mayta *et al.* [14]. It was synthetized in Metabion, Germany.

Amplification of DNA: PCR method was able to amplify DNA using Taq PCR Master Mix Kit. in Biometra T1 Thermocycler according to the instruction of the manufacturer.

PCR was performed using the following temperature profile: an initial denaturation at 94°C for 5 min followed by 30 cycles consisting of 94°C for 1 min (denaturation), 56°C for 1 min (annealing), 72°C for 2 min (elongation) and a final 72°C for 10 min as a final extension step. Ten microliters of PCR product was separated by electrophoresis on a 1.0% agarose gel containing 0.5 mg of ethidium bromide/ml to confirm the presence of amplification products.

The product was separated by horizontal electrophoresis in a 2.5% agarose gel stained with ethidium bromide and observation under short-wavelength ultraviolet radiation.

Biochemical Studies: Blood samples were collected by retro orbital venous plexus puncture and were used for serum separation. Serum samples stored at -20°C until further biochemical analyses. Determination of total proteins [15], albumin [16], activity of alanine aminotransferase (ALT) [17], total cholesterol [18] and creatinine [19] were determined. Test kits supplied by bioMérieux-France, were used.

Table 1: Synthetic PCR primer.

Primer design	Sequence	Ribosomal gene	Size of amplicons
TSS1 (F)	5′ GTCGTAACAAGGTTTCCGTA 3′	18S	
BD1 (R)	5′ATATGCTTAAGTTCAGCGGGTAATC 3′	28S	1,300 bp

(F): Forward. (R): Reverse. bp: base pair.

Statistical Analysis: All data were subjected to statistical analysis including the calculation of the mean and standard error (mean±SE). Significance between data of the biochemistical parameters in control and infested groups was evaluated by Student *t*-test at level $P<0.05$ [20] using SPSS for windows version 15 computer programme.

RESULTS

Experimental Infection of Balb/c Mice with *T. Saginata* Oncospheres: From fifteen inoculated mice with hatched oncospheres of *T. saginata*, 4 animals were positive for cysticerci of *Taenia saginata*.

The infected mice with *Taenia saginata* cysticercosis were also confirmed by PCR. The number of cysts collected from the BALB/c mice after 20 weeks post-infection ranged from 1 to more than 5 per mouse. Therefore, the BALB/c mice were used for further vaccine study. Viable mature cysticerci (tumor-like cysts) were recovered from the inoculation sites at 16 weeks post-infection.

Histopathological Examination: Histpathological examination of Semi-thin section of myocardium of mice inoculated with *T. saginata* oncospheres showed mononuclear leukocytic infiltration (red arrow) as well as aggregation in focal manner (Figure 1).

PCR Assay for Diagnosis of *Taenia Saginata* Cysticerci: PCR amplification with primers DB1 and TSS1 resulted in the detection of a single specific band of approximately 1,300 bp (Figure 2).

Serum Biochemical Changes: There was no significant difference in total serum proteins and albumin levels in both control non-infested mice and infested group with

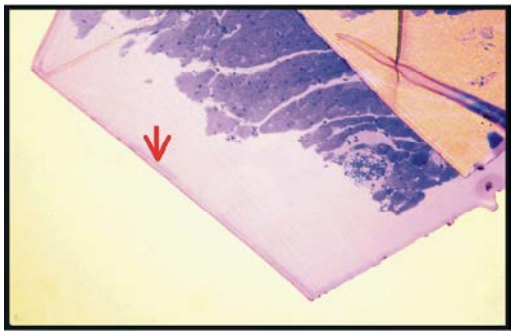

Fig. 1: Semi-thin section of myocardium of mice after 5 month from inoculation with *T. saginata* oncospheres showing mononuclear leukocytic inflammatory cells (red arrow) infiltrating the myocardium as well as aggregation in focal manner (H&E X20).

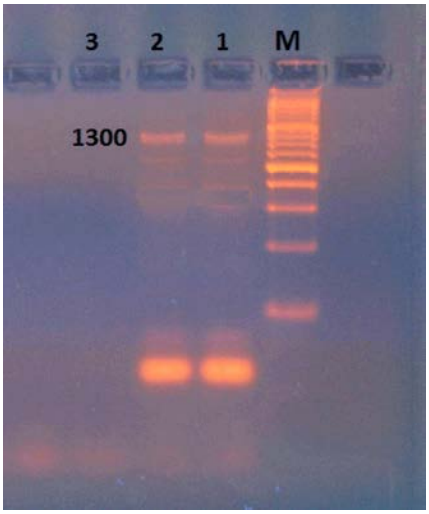

Fig. 2: The PCR products were resolved by 2.5% (w/v) agarose gel electrophoresis and visualized by ethidium bromide staining. On the right indicate the sizes (in bases pairs, bp) of the molecular weight markers. Lane (1-2): cyst of T. saginata. Lane (3): -ve Control. The PCR product of the cyst was 1.300 pb.

Table 2: Biochemical changes in serum of control and infected groups of Balb/c mice infected with *Taenia saginata* oncospheres (Mean ± SE, N=6).

Groups Parameters	Control (non-infested)	Infested
Total proteins (g/dl)	5.47±0.33	5.87±0.16
Albumin (g/dl)	3.94±0.23	3.77±0.07
Globulin (g/dl)	1.53±0.12	2.10±0.20*
A/G ratio	2.62±0.10	1.93±0.25*
Creatinine (mg/dl)	0.97±0.04	1.26±0.04**
Alanine aminotransferase(IU/l)	66.00±2.42	132.62±8.27**
Total Cholesterol (mg/dl)	86.00±5.51	118.55±4.47**

* = significant at $P \leq 0.05$. ** = Highly significant at $P \leq 0.01$. SE = Standard error A/G = Albumin/Globulin

T. saginata eggs. However, significant ($P<0.05$) increase in serum globulins (2.10±0.20 mg/dl) was recorded in infested group compared to non-infested group (1.53±0.12 mg/dl). Moreover, marked decrease ($P<0.05$) in A/G ratio (1.93±0.25) in infested group than control ones (2.62±0.10) (Table 2).

In serum of infested group of mice with *T. saginata* eggs, creatinine level (1.26±0.04 mg/dl), the activity of ALT (132.62±8.27 IU/l) and total cholesterol (118.55 mg/dl ±4.47) and were markedly ($P<0.01$) increased in comparison with the control non-infested group (0.97±0.04 g/dl, 66.00 ±2.42 IU/l and 86.00 ±5.51 mg/dl, respectively) (Table 2).

DISCUSSION

The present study showed that Balb-c mice become supreme experimental animal model for cysticercosis. Pigs and cattle have been primarily used for studying Taenia tapeworm infections [21, 22]. Because of the difficulty in handling these large animals, immunesuppressive mice were first introduced for studying these infections by Machnicka and Smyth [23]. *T. solium* Cysticercosis, *T. saginata asiatica* and *T. saginata* was successfully established in SCID mice by Ito *et al.* [6, 24] and Ito and Ito [25]. Furthermore, Wang *et al.* [12] inoculated 4 strains of normal mice, namely, ICR, BALB/cAnN, C57BL/6N and C3H/HeN, each with 1000 active *T. solium* or *T. saginata asiatica* oncospheres to assess the possibility of cysticerci formation. No satisfactory result was obtained with this dosage of oncospheres.

In this pilot experimental study, the cysticerci recovery rate of the BALB/c mice was high. Viable cysticerci were found in the BALB/c mice from the 8th week to the 20th week post-infection. The difference between the results of this study and those of the previous study might be due to genetic differences between the mice used in these two studies. Genetic factors play an important role in susceptibility to *T. crassiceps* model [26-28]. Further, the results of this study indicated that the female mice were suitable for developing an experimental model of oncosphere infection. Morales-Montor and Larralde [29] indicated that sex steroids were affected by the infection of Taenia.

Histpathological examination of infected mice heart revealed myocarditis. This result was similar with that of Gracey and Collins [10].

The identification of cysticerci had been confirmed by DNA analysis. PCR amplification with primers DB1 and TSS1 resulted in the detection of a single specific band of approximately 1,300 bp. Similar results were obtained by Rishi and McManus, [30], Flisser *et al*. [31], Harrison *et al*. [32] and Chapman *et* al. [33].

Large amount of *Cysticercus bovis* may cause hepatic dysfunction [34]. The most remarkable change was a rise in globulin in infested group of mice. It may be due to increase in γ- globulin which appears to be in response to the antigenic stimulation of the infectious agent, kidney damage, or myocarditis [8, 35-37].

This study revealed that there were changes in the ALT activity, total cholesterol and creatinine of infested mice with *T. saginata* viable eggs. Total cholesterol revealed high concentration in serum of infested mice. Cholesterol may have a role in pathogenesis by helping the larvae to survive in the host tissues or it may be due to the break in the liver function and changes the hormone secretion which provoked by the presence of parasite. Cholesterol enhanced larval survival, development and growth when added to RPMI-1640 culture medium and there may be some factors or enzymes, which allow the parasite to breakup and consume lipid/cholesterol [8, 38-40].

CONCLUSION

In conclusion, female BALB/c mice can be used as experimental animals for developing the *T. saginata* infection in vaccination studies. As well as *T. saginata* cysticercosis caused an alteration in liver and kidney functions.

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REFERENCES

1. Flisser, A., D. Correa, G. Avilla and P. Marvilla, 2005. Biology of *Taenia solium*, *Taenia saginata* and *Taenia saginata asiática*. In: Murrel. K.D. (Ed.) WHO/FAO/OIE guidelines for the surveillance, prevention and control of taeniosis/cisticercosis. Paris, France, pp: 1-8.
2. McFadden, A.M.J., D.D. Heath, C.M. Morley and P. Dorny, 2011. Investigation of an outbreak of *Taenia saginata* cysts (Cysticercusbovis) in dairy cattle from two farms. Vet. Parasitol., 176: 177-184.

3. White, A.C. and P.F. Weller, 2008. Cestodes. In: Kasper, D.L., Braunwald, E., Fauci, A.S., Hauser, S.L., Longo, D.L., Jameron, J.L., Loscalzo, j. (Eds). Harrisons Principles of internal Medicine, 17th edition. McGraw-Hill Medical publishing Dvision, New York, pp: 204.
4. Fan, P.C., C.Y. Lin, W.C. Chung and C.C. Wu, 1996. Experimental studies on the pathway for migration and the development of Taiwan Taenia in domestic pigs. Int. J. Parasitol., 26: 45-48.
5. Villa, O.F. and R.E. Kuhn, 1996. Mice infected with the larvae of Taenia crassiceps exhibit a Th2-like immune response with concomitant anergy and downregulation of Th1-associated phenomena. Parasitol., 112: 561-570.
6. Ito, A., W.C. Chung, C.C. Chen, M. Ito, S. Endo, M. Okamoto and P.C. Fan, 1997. Human Taenia eggs develop into cysticerci in SCID mice. Parasitology 114: 85-88.
7. Ito, A., K. Nakaya, Y. Sako, M. Nakao and M. Ito, 2001. NOD-SCID mouse as an experimental animal model for cysticercosis. Southeast Asian J. Trop. Med. Puplic Health, 32: 85-89.
8. Kandil, Omnia, M., Soad, M. Nasr, Mona, S. Mahmoud, Somia, A. Nassar, T.M. El-Metanawey, M.H.M. Abd El-Aziz and Nadia Abu El Ezz, 2012. Serological and biochemical studies on cattle naturally infested with *Taenia saginata* cysticercosis. Global Veterinaria, 9(5): 571-579.
9. Yan, L., Z. Wujun, B. Huai'en and C. Yan, 2007. Observations on pathological and histochemical changes in piglet livers infected with *Taenia saginata* asiatica. Frontiers Med. China, 1(3): 258-263.
10. Gracey, J.F. and D.S. Collins, 1992. Meat Hygiene. 9th ed. London: BaillièreTindall, pp: 143-167.
11. Ito, A., T. Yamada and T. Ishiguro, 1996. Vaccination of rats with frozen eggs, etanol-fixed eggs and frozen oncospheres with or without embryophoric blocks of *Taenia taeniaeformis*. Jpn. J. Parsitol., 45: 330-332.
12. Wang, I.C., Y.X. Ma, C.H. Kuo and P.C. Fan, 1997. A comparative study on egg hatching methods and oncosphere viability determination for Taenia solium eggs. Int. J. Parasitol., 27: 1311-1314.
13. NRC (National Research Council), 1995. Nutrient requirements of laboratory animals. Fourth Revised Edition, National Academy Press, Washington, D.C.
14. Mayta, H., A. Talley, R.H. Gilman, J. Jimenez, M. Verastegui, M. Ruiz, H.H. Garcia and A.E. Gonzalez, 2000. Differentiating *Taenia solium* and *Taenia saginata* infections by simple hematoxylin-eosin staining and pcr-restriction enzyme analysis. J. Clin. Microbiol, 38(1): 133-137.
15. Henary, R.J., D.C. Cannon and J.W. Winkleman, 1974. Clinical Chemistry Principles and Techniques.2nd Ed. Harper and Roe, New York.
16. Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chim. Acta, 31: 87-96.
17. Reitman, S.M.D. and S. Frankel, 1957. A colorimeter method for determination of serum glutamic oxaloacetic acid and glutamic pyruvic acid transferases. Am. J. Clin. Pathol., 28: 56-63.
18. Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.
19. Houot, O. 1985. Interpretation of Clinical Laboratory Tests.Ed. by Siest, G., Henny, J., Schiele, F. and Young, D.S. Biochemical Publications, pp: 220-234.
20. Petrie, A. and P. Watson, 1999. Statistics for Veterinary and Animal Science. 1st Ed., the Blackwell Science Ltd., U.K., pp: 90-99.
21. Urquhart, G.M. and D.W. Brocklesby, 1965. Longevity of Cysticercus bovis. J. Parasitol., 51: 349.
22. Heinz, H.J. and L. Aron, 1966.Studies on Cysticercus cellulosae. S. Afr. J. Med. Sci., 31: 61-66.
23. Machnicka, B.S. and J.D. Smyth, 1985. The early development of larval *Taenia saginata in vitro* and *in vivo*. Acta Parasitolica Polonica, 30: 47-52.
24. Ito, A., M. Ito, K.S. Eom, W.C. Chung, C.C. Chen, L. Ma, S. Enod and P.C. Fan, 1997. In vitro hatched oncospheres of Asian taenia from korea and Taiwan develop into cysticerci in the peritonial cavity of female SCID (severe combined immunodeficiency) mice. Int. J. Parasitol., 27: 631-633.
25. Ito, M. and A. Ito, 1999. Human Taenia in severe combined immunodeficiency (SCID) mice. Parasitol. Today, 15: 64-67.
26. Fragoso, G., E. Lamoyi, A. Mellor, C. Lomeli, T. Govezensky and E. Sciutto, 1996. Geneticcontrol of susceptibility to Taenia crassiceps murine cysticercosis. Parasitol., 112: 119-124.

27. Fragoso, G., E. Lamoyi, A. Mellor, C. Lomeli, M. Hernandez and E. Sciutto, 1998. Increased resistance to *Taenia crassiceps* murine cysticercosis in Qa-2 transgenic mice. Infect. Immun., 66: 760-764.
28. Lopez-Briones, S., E. Lamoyi, G. Fragoso, M.J. Soloski and E. Sciutto, 2003. Taenia crassiceps cysticercosis: immune response in susceptible and resistant BALB/c mouse substrains. Parasitol. Res., 90: 236-242.
29. Morales-Montor, J. and C. Larralde, 2005. The role of sex steroids in the complex physiology of the host-parasite relationship: the case of the larval cestode of Taenia crassiceps. Parasitol., 131: 287-294.
30. Rishi, A.K. and D.P. McManus. 1987. Genomic cloning of human *Echinococcus granulosus* DNA: isolation of recombinant plasmids and their use as genetic markers in strain characterization. Parasitology, 94: 369-383.
31. Flisser, A., A. Reid, E. Garcia-Zepeda and D.P. McManus. 1988. Specific detection of Taenia saginata eggs by DNA hybridization. Lancet ii, pp: 1429-1430.
32. Harrison, L.J.S., J. Delgado and R.M.E. Parkhouse. 1990. Differential diagnosis of *Taenia saginata* and *Taenia solium* with DNA probes. Parasitol., 100: 459-461.
33. Chapman, A., V. Vallejo, K.G. Mossie, D. Ortiz, N. Agabian and A. Flisser.1995. Isolation and characterization of species-specific DNA probes from *Taenia solium* and *Taenia saginota* and their use in egg detection assay. J. Clin. Microbiol, 33: 1283-1288.
34. Scandrett, B., S. Parker, L. Forbes, A. Gajadhar, P. Dekumyoy, J. Walkagul and D. Haines, 2009. Distribution of *Taenia saginata* cysticerci in tissues of experimentally infected cattle. Vet. Parasitol, 164: 223-231.
35. Chapman, C.B., P.M. Knopf, J.D. Hicks and G.F. Mitchell, 1979. IgG1 hypergamma globulinaemia in chronic parasitic infections in mice: magnitude of the response in mice infected with various parasites. Aust. J. Exp. Biol. Med. Sci., 57(4): 369-387.
36. Coles, E.H., 1986. Veterinary Clinical Pathology. 4th Ed. published by W.B. Saunders Co., Philadelephia.
37. Thrall, M.A., D.C. Baker, T.W. Campbell, D. DeNicole, M.J. Fettman, E.D. Lassen, A. Rebar and G. Weiser, (Eds.), 2004. Veterinary Hematology and Clinical Chemistry. Lippincott Williams and Wilkins, Philadelphia, pp: 518.
38. Urban, J.F., F.W. Douvres and S. Xu, 1984. Culture requirement of Ascaris suum larvae using a stationary multi-well system: increased survival, development and growth with cholesterol. Vet. Parasitol., 14: 33-42.
39. Biadun, W., 1990. Studies of serum lipids in Guinea pigs with larval ascariasis. Wiad Parazytol., 36: 15-26.
40. Wiedermann, U., H. Stemberger, E. Unfried, K. Widhalm, M. Kundli, M. Altenriederer, M. Savedra and G. Wiedermann, 1991. Intestinal worm burden and serum cholesterol or lipid concentration in a Shipibo population (Peru). Zentrabl Bakteriol, 275: 279-286.