Global Veterinaria 9 (5): 571-579, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.5.66230

Serological and Biochemical Studies on Cattle Naturally Infested With *Taenia saginata* Cysticercosis

[1]*Omnia M. Kandil,* [1]*Soad M. Nasr,* [1]*Mona S. Mahmoud,* [1]*Somia A. Nassar,*
[1]*T.M. El-Metanawey,* [2]*M.H.M. Abd El-Aziz and* [1]*Nadia M.T. Abu El Ezz*

[1]Department of Parasitology and Animal Diseases,
El-Behouse Street, Dokki, Post Box, 12622,Giza, Egypt
[2]Department of Molecular Biology, National Research Center,
El-Behouse Street, Dokki, Post Box, 12622,Giza, Egypt

Abstract: Diagnosis of *Taenia saginata* (*T. saginata*) cysticercosis in naturally infected cattle by enzyme linked immunosorbent assay (ELISA) is the main purpose of the current study. Also determination of *Cysticercus bovis* (*C. bovis*) effect on haematology and serum biochemistry was performed as additional approach for diagnosis. Blood samples were collected from 75 slaughtered cattle at El-Basateen abattoir. *Post-mortem* examination of slaughtered cattle showed 4% infected cases with *T. saginata* cysticerci. The results of ELISA revealed 29. 3% seropositive samples with *C. bovis* using crude antigen. Optical density (OD) values of positive investigated samples were classified into three categories; high (6. 7%), moderate (4%) and low (18. 7%). The crude extract was fractionated on Diethyl-Aminoethyl (DEAE) cellulose column. Two partially purified antigens were obtained [P1=unadsorbed (-ve) and P2=adsorbed (+ve)]. Electrophortic separation of crude antigen of *C. bovis* showed 13 bands with molecular weight starting from 235 to 14 kDa. While, P1 and P2 had 5 and 8 bands, respectively. Western immunoblotting of separated antigens against hyperimmune serum developed only one band at 57 kDa. The results revealed that there was no effect on haematological parameters while, there was a significant decrease in total serum proteins (P<0.05) of infested cattle. High levels of albumin and α1-globulin in infested cattle were recorded compared to non-infested group. However, in all seropositive categories, marked (P<0.05) increase in γ - globulin levels than non-infested cattle was observed. The activity of Aspartate amino transferase (AST) in serum was markedly (P<0.05) decreased in low infested cattle compared to non-infected. In all groups of infestation, total serum cholesterol and urea levels were decreased (P<0.05) particularly in highly infested cattle. On the other hand, creatinine level was increased significantly (P<0.05) in serum of highly infested cattle compared to non-infested ones. In conclusion, performance characteristics of ELISA suggest its field applicability in bovine cysticercosis outbreaks. As well as alteration in liver and kidney functions could be considered a marker for *T. saginata* cysticercosis in cattle.

Key words: Cattle · *Taenia saginata* · Cysticercosis · ELISA · Serum biochemistry diagnosis

INTRODUCTION

T. saginata is a medically and economically important cestode parasite. Cattle become infested as intermediate hosts after eating *T. saginata* eggs (proglottids) from infected humans. Cysticerci develop in the muscle of cattle and subsequently become infective to humans after approximately 10 weeks [1, 2]. *T. saginata* cysticercosis is found worldwide but causes the greatest economic impact and public health implications in tropic and sub-tropic regions [3]. In the meat industry, economic losses are closely associated with the status of infection. If a heavy infection or generalized cysticercosis is found in a carcass, it must be totally condemned, Light infection or localized cysticercosis leads to condemnation of the infected parts; furthermore, the carcass must be kept

Corresponding Author: Omnia M. Kandil, Department of Parasitology and Animal Diseases, Veterinary Research Devision, National Research Centre, Postal code: 12622, Dokki, Giza, Egypt.

in cold storage at a temperature not exceeding -70°C for up to 3 week to inactivate the parasites [4-6]. Immunodiagnosis provide practical means of specific pre-mortem examination of larval cestode infections[7]. ELISA is sensitive but its specificity depends on the utilized antigen [8]. Antibody detecting ELISA based on crude antigen preparations showed high efficiency of cysticercosis diagnosis [9-11], but cross reactions continue to be a challenge. Both ELISA and immunoblotting may be considered useful tools for epidemiological studies of bovine cysticercosis [12, 13].

T. saginata asiatica cysticerci damaged host (pig) liver tissues leads to the metabolic disorder of lipid, glycogen, protein and changes in enzyme metabolism [14]. In cattle, heavy infestation by *T. saginata* cysticerci may cause myocarditis and heart failure [15]. However, few literatures are available concerning changes in serum biochemical parameters of cattle infested by *T. saginata* cysticerci.

The current study aimed to diagnose *T. saginata* cysticercosis in slaughtered cattle by detecting IgG antibody using ELISA. Detection of changes in haematological and biochemical parameters as a result of infection was also undertaken.

MATERIALS AND METHODS

Cysticerci Samples: *T. saginata* cysticerci were obtained from slaughtered cattle at El-Basateen abattoir, Cairo, following meat inspection at specific predilection sites (external and internal masseter muscles, tongue, heart and diaphragm) as previously mentioned by Lopes *et al*. [16].

Blood Samples: Blood samples were collected from 75 slaughtered cattle at El-Basateen abattoir, Cairo. Two blood samples were collected from each animal. The first sample was collected on ethylene diamine tetra-acetic acid (EDTA) for hematological evaluation. The second blood sample was placed in a plain centrifuge tube allowed to clot for serum separation and stored at -20°C until use in serological and biochemical analyses. Three cattle sera proved to harbor *C. bovis* only as indicated by their postmortem examination after slaughtering were used as positive control.

Preparation of Crude Antigen: Crude antigen of whole *C. bovis* cysts was prepared by homonization and sonication of cysts in phosphate buffer saline (PBS) according to the method of Lightowlers *et al*. [17]. The protein content was estimated using the method of Lowery *et al*. [18].

Preparation of Hyperimmune Serum: The hyperimmune serum was prepared by injecting *C. bovis* antigen in three New Zealand white rabbits free from parasite according to the protocol done by Aswai *et al*. [19]. Briefly, each rabbit was subcutaneously injected with 1 ml (200µg protein /kg) of the antigen emulsified with equal volume (v/v) of complete freund's adjuvant. Booster doses of antigen emulsified with incomplete freund's adjuvant were given one and two weeks later, one week after the last booster dose, rabbit blood samples were collected and sera were separated and stored at -20°C.

Antigen Purification: The crude extract was applied on DEAE-cellulose column previously equilibrated. After sample application, the column was washed with equilibration buffer and the adsorbed proteins were eluted using 0. 2 M NaCl in the equilibration buffer. The two fractions were collected at a flow rate of 60 ml/h and designated P1 (unadsorbed, - ve) and P2 (adsorbed, + ve) according to their elution order following Abdalla *et al*. [20]. The protein concentrations of the fractions were determined by Lowery *et al*. [18].

Enzyme-Linked Immunosorbent Assay (ELISA): The potency of *C. bovis* antigen was evaluated by ELISA which was performed according to Zimmerman *et al*. [21]. The optimal antigen, serum and conjugate concentrations were determined after preliminary checkerboard titration according to Catty and Raykundalia [22]. The cyst antigen concentration was 5 µg/ml. After coating, blocking with 100 µL per well of 0. 1% bovine serum albumin in 0.01 M PBS was done. From diluted cattle sera (diluted 1:800 in PBS), 100 µL were added to each well. 100 µl of 1:1000 peroxidase conjugate anti-bovine IgG were used. 50 µl of Ortho-phenylenediamine was used as a substrate. The reaction was terminated with 1M H_2SO_4 and the absorbance values were read spectrophotometrically at 405 nm. Positive samples were assigned according to Rodriguez-Peiez and Hillyer [23] as those with absorbance readings greater than the cut off value, which was calculated as mean OD of negative sera plus three standard deviations.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE): Ten mg of crude and fractions of *C. bovis* antigen was electrophoresed using 10% SDS- PAGE under reducing condition [24]. Gels were stained by Commmassie blue stain with several changes in destaining solution till the bands became clear.

Western Blotting Technique: The immunogenicity of crude antigen and isolated fractions of *C. bovis* was evaluated by immunoblot. After SDS-PAGE, protein bands were electroblotted on nitrocellulose paper according to Towbin *et al*. [25] in a blotting system and their immunogenicity was evaluated against hyperimmune serum. Molecular weight of detected bands was calculated according to the software analysis gel pro-analyzer.

Clinicopathological Studies

Haematological Investigations: Packed cell volume (PCV%), Haemoglobin concentration (Hb), Red blood cell counts (RBCs) and total leukocytic counts were investigated in infested and non infested cattle according to Feldman *et al*. [26].

Serum Biochemical Studies

Serum Proteins Profile: Determination of total proteins was performed according to the method of Henary *et al*. [27] the used test kit was supplied by bioMérieux, France. Electrophoresis was performed using a Semi-automated agarose gel electrophoresis system (Helena Laboratories, Helena Biosciences, Gatesheade, UK) according to the manufacturer's instructions. Using the computer software Phoresis (Helena Biosciences), electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentrations (g/dL) were calculated using the total serum protein concentration.

Serum Enzymes, Total Cholesterol, Urea and Creatinine Profiles: Aspartate amino transferase (AST) and alanine amino transferase (ALT) activities [28], total cholesterol [29], urea [30] and creatinine [31] were determined using test kits supplied by bioMérieux-France.

Statistical Analysis: All data were subjected to statistical analysis including the calculation of the mean and standard error (mean±SE). Significance between data of haematological parameters in control and infested groups were evaluated by Student *t*-test at level $P<0.05$. However, differences between data of biochemical parameters in control and degrees of infested groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered significant at $P<0.05$ level [32] using Statistical Package for Social Science (SPSS) for windows version 15 computer program.

RESULTS

Sensitivity of ELISA: Sensitivity of ELISA compared with *post-mortem* examination is shown in table 1. Meat inspection revealed 4% infected cases while, 29. 3% positive cases were detected by ELISA. The low, moderate and high degrees of infection occurred in 18. 7%, 4% and 6. 7% of examined samples respectively (Table 2).

Electrophoretic Profile of *T. Saginata* Antigens: Electrophoresis separation of crude antigen of *C. bovis* showed 13 bands starting from 235 to 14 kDa.

Table 1: Detection of anti-*C. bovis* antibodies in sera from slaughtered cattle compared with meat inspection findings

	Meat inspection				ELISA			
	Positive		Negative		Positive		Negative	
No. of examined cattle	No.	%	No.	%	No.	%	No.	%
75	3	4. 0	72	96. 0	22	29. 3	53	70. 7

Table 2: Different degrees of seropositive cattle infected with *C.bovis* using ELISA

					ELISA							
	Meat inspection				Positive							
	Positive		Negative		Low		Moderate		High		Negative	
No. of examined cattle	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
75	3	4. 0	72	96. 0	14	18. 7	3	4. 0	5	6. 7	53	70. 7

Table 3: Different molecular weights (KDa) of *C. bovis* bands; crude antigen (C) and purified antigens (P1 & P2) by SDS-PAGE.

P1	P2	C	Standard
	235	235	260
	163	163	140
90		111	100
	84	84	70
66	66	66	50
48	48	48	40
		41	35
		36	25
	30	30	15
22	22	22	10
20	20	20	
		12	
		14	

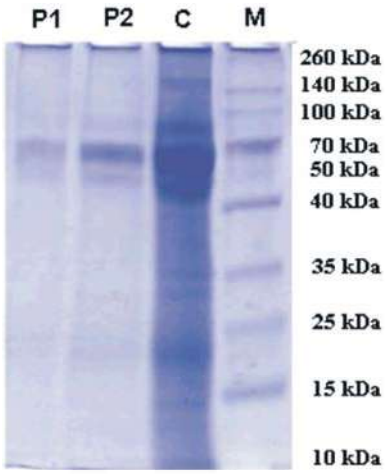

Fig. 1: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of *Cysticercus bovis*; crude antigen (C) and purified antigens (P1 & P2) was developed in comparison to broad range molecular weight marker (M).

While, two purified antigens P1 and P2 obtained from DEAE cellulose column had 5 and 8 bands respectively. There were four common bands between the three antigens (crude, P1 and P2) of molecular weight 66, 48, 22 and 20 KDa (Table 3 and Fig. 1).

Immunogenic Bands: Western immunoblotting of separated antigens when reacted against hyperimmune serum in the present study had developed only one band at 57kDa (Fig. 2)

Clinicopathological Findings

Haematological Findings: There was no significant difference in PCV%, haemoglobin, RBCs and WBCs in both control (non-infected) and infested group with *C. bovis* (Fig. 3).

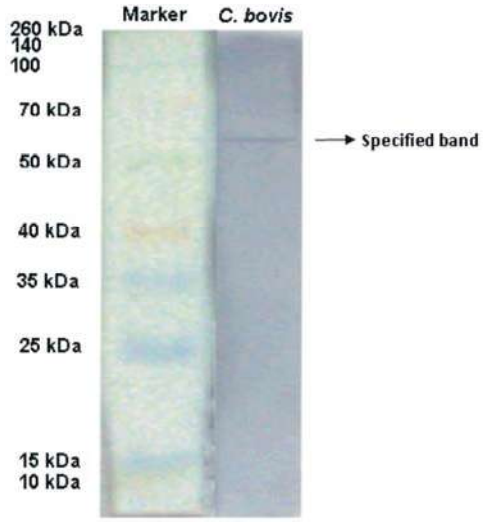

Fig. 2: Western immunoblotting pattern of examined *C. bovis* proteins against hyperimmune serum.

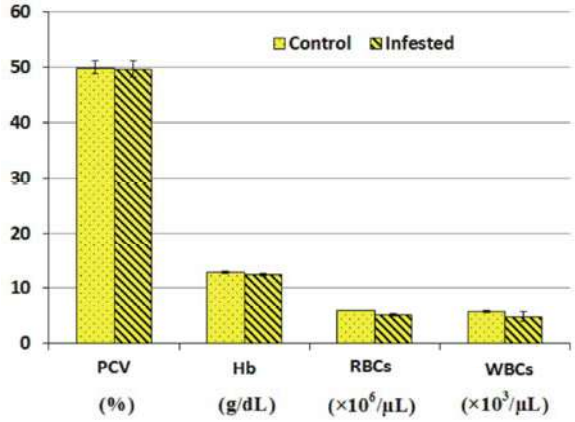

Fig. 3: PCV, Hb, RBCs and WBCs in control (Non-infested) and infested groups with *C. bovis*. (Mean ±SE, N=5).

Serum Biochemical Changes

Serum Proteins Profile: There was a significant (P<0.05) decrease in total serum proteins and albumin levels of highly infested cattle with *C. bovis* compared to non-infested group. However, significant (P<0.05) increase in total serum proteins was recorded in low infested cattle compared to non-infested group. Groups of moderate and high degree of infestation revealed marked decrease in α1- globulin than the control group. While, β-globulin level in moderate infested group showed marked (P<0.05) increase compared to high degree of infested cattle. The concentration of γ- globulin was elevated in all groups of infestation. While, no significant changes were noticed in α2- globulin and A/G ratio values between all groups (Fig. 4).

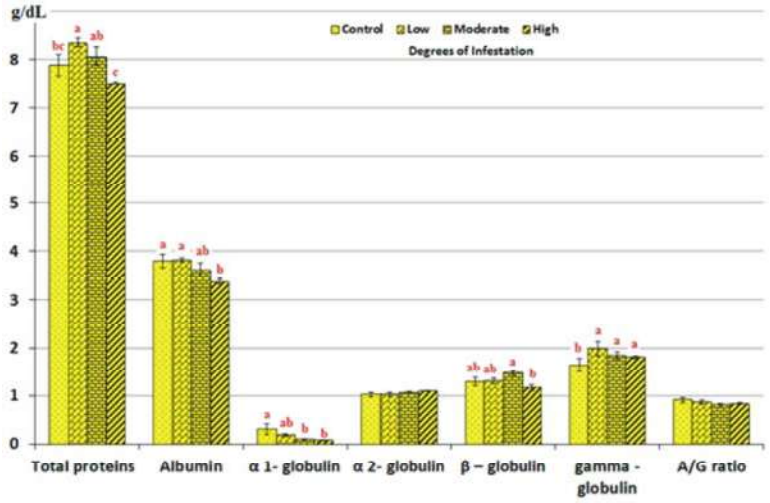

Fig. 4: Serum proteins profile in control (Non-infested) and infested groups with *C. bovis*. (Mean ±SE, N=10). Means with different superscript (a, b, c) in each parameter are significantly different at $P<0.05$.

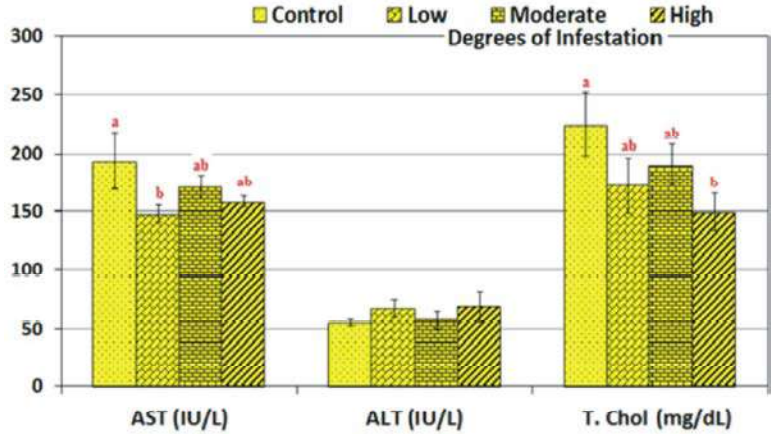

Fig. 5: Aspartate amino transferase (AST), alanine amino transferase (ALT) activities and total cholesterol (T. Chol) in serum of control (Non-infested) and infested cattle with *C. bovis* (Mean ± SE, N=10). Means with different superscript (a, b, c) in each parameter are significant different at $P<0.05$.

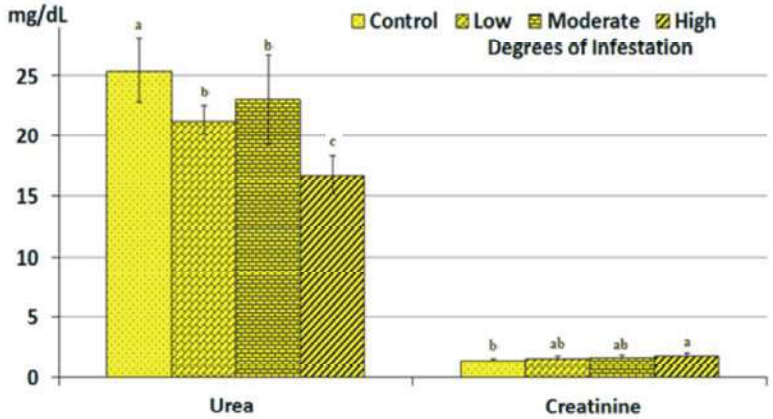

Fig. 6: Urea and creatinine levels in serum of control (Non-infested) and infested cattle with *C. bovis* (Mean ± SE, N=10). Means with different superscript (a, b, c) -in each parameter- are significant different at $P<0.05$.

Serum Enzymes, Total Cholesterol, Urea and Creatinine: In low infested cattle with *C. bovis*, the activity of AST in serum showed marked (P<0.05) decrease in comparison with control non-infested group. While, the activity ALT in serum revealed no significant differences between all groups (Fig. 4). In all groups of infestation, total serum cholesterol and urea levels were decreased which was obviously (P<0.05) in high degree of infested cattle compared to control group. On the other hand, creatinine level was increased significantly (P<0.05) in serum of highly infested cattle compared to control ones (Fig. 5 & 6).

DISCUSSION

In the present study, the occurrence of *C. bovis* was 4% using meat inspection which reported among 75 examined cattle in Egypt. This finding is considered higher than previously recorded by Hariday *et al*. [33] (0. 23%), Rodriguez- Hidalgo *et al*. [34], (0. 37%) and Abdo *et al.* [35] (1. 65%). On the contrary, the occurrence of *C. bovis* in this study is lower than those reported by Oryan *et al*. [36] (7. 7%), and Kandil *et al*. [37] (4.4%). The variation in the reported prevalence rates are expected matter due to several factors such as climatic variations between the localities, number of the collected samples as well as control measures and eradication programs in such countries.

In the present study, it was found that 29. 3 % of examined cattle were seropositive for *C. bovis* using crude antigen in ELISA. Adoption of ELISA for testing large numbers of animals was previously documented [10]. Crude antigen was utilized in ELISA for antibodies detection in serum of natural infected cattle with *C. bovis*. ELISA was used for detection of the recent infection which is not yet visible at slaughter or at postmortem [10]. Our results were in agreement of kandil *et al*. [37] they showed that 29.4% and 61.76% of examined sera were positive by using *C. bovis* and *T. saginata* crude antigens respectively.

Electrophoresis separation of crude antigen of *C. bovis* showed 13 bands starting from 235 to 14 kDa. While, two purified antigens P1 and P2 obtained from DEAE Cellulose column had 5 and 8 bands respectively. There were four common bands between the three antigens (crude, P1 and P2) with molecular weight of 66, 48, 22 and 20 KDa. Kandil *et al*. [38] found that the crude *C. bovis* antigen contains 12 bands and the shared bands between our results were 84, 48, 41, 36 and 20 kDa. *C. bovis* and *T. saginata* crude antigens revealed at least 10 bands for each antigen. The molecular weights of the separated bands ranged from 18-276 kDa. Two common bands (45 and 73 KDa) were major bands in *C. bovis* crude antigen, while all of them were major bands in *T. saginata* crude antigen [37]. Indeed, Ralston and Heath [39] described an immunoblot test for antibodies to *T. ovis* based on the recognition of a 94-kDa antigen band in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile of *T.* ovis *scolex* secretions. These variations might be related to the species as well as the type of antigen used.

Western immunoblotting of separated antigens when reacted against hyperimmune serum in the present study had developed only one band at 57kDa. Oliveira, *et al*. [7] used *C. bovis* antigen in immunoblot assay to diagnose neurocysticercosis (NC) and detected two bands at molecular weight of 64 and 68 KDa. They suggested that the antigenic fractions without affinity to ConA, obtained from *T. saginata* metacestodes, are an important source of specific peptides and are efficient in the diagnosis of NC when tested by immunoblot assay. Further investigations will be essential to isolate the immune- reactive antigens and to evaluate their potency in protection against cysticercosis infection.

There was no statistical difference in PCV%, haemoglobin, RBCs and WBCs in both control (non-infected) and infected group with *C. bovis*.

Large amount of *C. bovis* may cause hepatic dysfunction [40]. The most remarkable changes in serum protein were a rise in γ-globulin and a fall in albumin especially in high infested group of cattle. In the present study, the increase in γ-globulin appears to be in response to the antigenic stimulation of the infectious agent; the decrease in albumin may resulted from severely-depressed food intake, hemorrhage, kidney damage, or myocarditis [41-43]. A similar elevation in serum globulin concentration with an associated decrease in serum albumin concentration in calves experimentally infected with *T. saginata* was reported by Gallie and Sewell [44] and by Evranova and Mosina [45]. The latter authors found that the increase in the globulin concentration involved increases in the β- and γ- globulin fractions.

The present study revealed that there were changes in the activities of AST and ALT, and levels of total cholesterol, urea and creatinine of infested cattle with *C. bovis*. The decrease in the activity of AST in infected cattle may be due to the greater amount of cysts and subsequent chronic destruction of the hepatic parenchyma [46, 47]. In addition, a decrease in the levels of serum urea probably due to the observed hepatic damage in animals, thus the ability of the liver to convert

ammonia to urea was affected. Total cholesterol revealed low concentration in serum of infested cattle. It may be due to the role of cholesterol in pathogenesis by helping the larvae to survive in host tissues or it may be due to the break in the liver function and changes in the hormonal secretion which provoked by the presence of parasites. Cholesterol enhanced survival of ascariasis larval 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 [48-50].

CONCLUSION

It was concluded that this study pointed to the usefulness of ELISA for diagnosis of *T. saginata* cysticercosis in live cattle and evaluate the reliability of meat inspection procedure for detecting cysticercosis in carcasses of cattle. As well as *T. saginata* cysticercosis caused an alteration in liver and kidney functions.

ACKNOWLEDGMENT

This work was supported by the scientific project funded from the National Research Center No. S-91203

REFERENCES

1. Flisser, A., D. Correa, G. Avilla, and P. Marvilla, 2005. Biology of *Taenia solium*, *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 out break of *Taenia saginata* cysts (Cysticercusbovis) in dairy cattle from two farms. Vet. Parasitol., 176: 177-184.
3. Basem, R.N.A., S.M.S. Amal, A.A.H. Asmaa and I.A. Mohsen, 2009. Occurrence of Cysticercosis in cattle and buffaloes and *Taenia saginata* in man in Assiut Governance of Egypt. Vet. World, 2: 173-176.
4. Kandil, O.M., Olfat, A. Mahdy, Sanaa, K. Abou-Dobal and W.M. Mousa, 2004. Purification and characterization of the three larval taeniid antigens by gel filtration. Vet. Med. J., 52: 449-456.
5. 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.
6. Ibrahim, N. and F. Zerihum, 2012. Prevalence of *Taenia saginata* cysticercosis in cattle slaughtered I Addis Ababa municipal abattoir, Ethiopia. Global Veterinaria, 8: 467-471.
7. Oliveira, H.B., G.A. Machado, J.R. Mineo and J.M. Costa-Cruz, 2010. *Taenia saginata* metacestode antigenic fractions without affinity to concanavalin A are an important source of specific antigens for the diagnosis of human neurocysticercosis. Clin Vaccine Immunol., 17: 638 -644.
8. Lijiang, A., W. Hao and I.H. Akira, 2001. Immunodiagnostic differentiation of alveolar and cystic echincoccosis using ELISA with 18-kDa antigen extracted from Echinococcus protoscoleces. Trans. R. Soc. Trop. Med. Hyg., 95: 285-288.
9. Ogunrinade, A.F. and O.D. Oyekole, 1990. Evaluation of the efficiency of beef inspection procedures for tuberculosis, fascioliasis and cysticercosis in a nigerian abattoir. Prev. Vet. Med., 8: 71-75.
10. Kandil, O.M., 2005. Evaluation of circulating and affinity purified *Cysticercus taenuicollis* antigens in diagnosis of sheep cisticercosis by using ELISA. Egypt. Vet. Med. Assoc., 65(1): 179-188.
11. Minozzo, J.C., V. Thomaz-Soccol, C.C. Olortegui, V.E. Soares and A.J. Costa, 2004. Testeimunoenzima´tico (enzyme-linked immunosorbent assay) paradiagno´stico da cisticercose bovina e estudo da cine´tica de produc¸a˜o de anticorpos contra- *Cysticercus bovis*. Cien. Rural., 34: 857-864.
12. Prestes-Carneiro, L.E., S.B. Freita, S.C. Zago, N.A. Miguel, O.B. Primo, A.H. Iha, N.M. Espíndola and A.J. Vaz, 2006. Taeniosis-cysticercosis complex in individuals of a peasants' settlement (Teodoro Sampaio, Pontal of Paranapanema, SP, Brazil). Mem. Inst. Oswaldo Cruz, 101: 15-20.
13. Oliveira, H.B., R.M. Rodrigues, I.S.C. Barcelos, L.P. Silva and J.M. Costa-Cruz, 2006. Anti-*Taenia solium* metacestode IgG antibodies in serum samples from inhabitants of a central-western region of Brazil. Rev. Inst. Med. Trop. Sao Paulo, 48: 49-52.
14. 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*. Front. Med. China, 1: 258-263.
15. Gracey, J.F. and D.S. Collins, 1992. Meat hygiene. 9th ed. London: BaillièreTindall, pp: 143-167.

16. Lopes, W.D., T.R. Santos, V.E. Soares, J.L. Nunes, R.P. Mendonça, de R.C. Lima, C.A. Sakamoto, G.H. Costa, V. Thomaz-Soccol, G.P. Oliveira and A.J. Costa, 2010. Preferential infection sites of *Cysticercus bovis* in cattle experimentally infected with *Taenia saginata* eggs. Res. Vet. Sci., 19: 20-26.
17. Lightowlers, M.W., R. Rolfe and C.G. Gauci, 1996. *Taenia saginata*: Vaccination against cysticercosis in cattle with recombinant oncosphere antigens. Exp. Parasito., 84: 330-338.
18. Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
19. Aswai, S.M., S.D. Carter and Z. Woldehiwet,1993. The isolation and characterization of camel (*Camelus dromedaries*) immunoglobuline classes and subclasses, J. Comp. Path., 109: 187-195.
20. Abdalla, A.M., M.A. El-Mogy, N.M. Farid and M.M. El-Sharabasy, 2006. Two glutathione S-transferase isoenzymes purified from Bulinus truncates (Gastropoda: Planorbidae). Comp. Biochem. Physiol., 143: 76-84.
21. Zimmerman, G.L., L.W. Jen, J.E. Cerro, K.L. Farnsworth and R.B. Wescott, 1982. Diagnosis of *Fasciola hepatica* infections in sheep by an enzyme linked immunosorbent assay. Am. J. Vet. Res., 43: 2097-2100.
22. Catty, D. and C. Raykundalia,1989. ELISA and related enzyme immunoassays. In: Catty, D. (Ed.), Antibodies, a Practical Approach, vol. II. IRL Press Oxford University Press, Oxford, pp: 97-152.
23. Rodriguez-Peiez, J. and G.V. Hillyer, 1995. Detection of excretory-secretory circulating antigens in sheep infected with *Fasciola hepatica* and with *Schistosoma mansoni* and *Fasciola hepatica*. Vet. Parasitol., 56: 57-66.
24. Laemmli, U.K., 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
25. Towbin, H., T. Staehelin and J. Gordon, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proceeding Natural Academic Science. USA, 176: 4350-4354.
26. Feldman, B.F., J.G. Zinkl and N.C. Jain, 2000. Scham`s Veterinary Hematology. 5th ed. published by Lea and Febiger, Philadelphia, USA.
27. Henary, R.J., D.C. Cannon and J.W. Winkleman, 1974. Clinical Chemistry Principles and Techniques. 2nd Ed. Harper and Roe, New York.
28. 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.
29. 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.
30. Patton, C.J. and S.R. Crouch, 1977. Calorimetric determination of urea. Analyt. Chem., 49: 464-469.
31. 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.
32. Snedecor, G. and C. William, 1989. Statistical Methods. 8th Ed., Ames, Iowa State University Press, pp: 217-235.
33. Haridy, F.M., B.B. Ibrahim, T.A. Morsy and I.M. El-Sharkawy, 1999. Human taenaisis and cisticercosis in slaughtered cattle, buffaloes and pigs in Egypt. J. Egypt . Soc . Parasitol., 29: 375-394.
34. Rodriguez- Hidalgo, R.F. Hamann and P. Hall, 2003. Taeniosis- cisticercosis in man and animals in the Sierra of Northern Ecuador. Vet. Parasitol., 118 : 51-60.
35. Abdo, B.R.N., A.S.M. Sayed, A.A.A. Hussein and M.I. Arafa, 2009. Occurrence of cisticercosis in cattle and buffaloes and *Taenia saginata* in man in Assuit governance of Egypt. Vet. World, 2: 173-176.
36. Oryan, A., N. Moghadder and S.N. Gaur, 1995. *Taenia saginata* cisticercosis in cattle with special references to its prevalence. Pathogenesis and economic implications in Fars Provice of Iran. Vet. Parasitol., 57: 319-327.
37. Kandil, O.M., M.S. Mahmoud, H.A. Shalaby, A.H. El Namaky, S.H.M. Hendawy and M.I. Arafa, 2012. Value of *Taenia saginata* Crude Antigen in Diagnosis of Bovine Cysticercosis with Reference to its Characterization. Global Veterinaria, 9: 474-478.
38. Kandil, O.M., M. Mahmoud, S. Mona and Khalil A. Fathia, 2003. Characterization and demonstration of species specific and cross-reactive components of cysticercosis and hydatidosis. J. Egypt. Vet. Med. Assoc., 63: 277-289.
39. Ralston, M.J. and D.D. Heath, 1995. A defined antigen for the serodiagnosis of *T. ovis* infections in dogs. J. Parasitol., 81: 422-428.

40. 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.
41. Chapman, C.B., P.M. Knopf, J.D. Hicks and G.F. Mitchell, 1979. IgG1 hyper-gamma globulinaemia in chronic parasitic infections in mice: magnitude of the response in mice infected with various parasites. Aust J. Exp. Biol. Med. Sci., 57: 369-387.
42. Coles, E.H.,1986. Veterinary Clinical Pathology. 4th ed. published by W. B. Saunders Co., Philadelephia
43. 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.
44. Gallie, G.J. and M.M.H. Sewell, 1974. The serological response of three month old calves to infection with *Taenia saginata* (cysticercus bovis) and their resistance to reinfection Trop. Anim. Hlth Prod., 6: 163-171.
45. Evranova, V.G. and S.K. Mosina, 1965. An electrophoretic picture of serum protein in experimental echinococcosis of sheep and cysticercosis of cattle. Materialy Nauctmoi Konferentsii Vsesoyuznogo Obshchestva Gelmintologov, Part, 4: 327-330. (In Russian)
46. Pinzani, M. and K. Rombonts, 2004. Liver fibrosis: from the bench to clinical targets. Dig. Liver Dis., 36: 231-242.
47. Otto, M.A., A. Da Silva, S.P. Wolkmer, C.K. Traesel, C. Schmidt, Tonin, A.A.R.A. Zanette, R.A. Lopes, S.T.A. and S.G. Monteiro, 2010. Levels of liver enzymes and urea in rats naturally infected with larval forms of *Taenia taeniformis*. Comp. Clin. Pathol., 19: 527-529.
48. Urban, J.F., F.W. Douvres and S. Xu, 1984. Culture requirement of Ascarissuum larvae using a stationary multi-well system: increased survival, development and growth with cholesterol. Vet. Parasitol., 14: 33-42.
49. Biadun, W., 1990. Studies of serum lipids in guinea pigs with larval ascariasis. Wiad Parazytol., 36: 15-26.
50. 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 Shipibopopulation (Peru). Zentrabl Bakteriol., 275: 279-86.