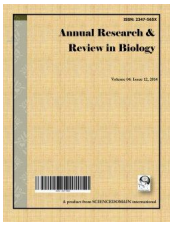

Annual Research & Review in Biology

26(5): 1-6, 2018; Article no.ARRB.41535
ISSN: 2347-565X, NLM ID: 101632869

Diagnostic Performance Characteristics of Serum Chemistry in the Detection of *Cysticercus fasciolaris* in Brown Rats (*Rattus norvegicus*)

Richard M. Kabuusu[1], Keshaw Tiwari[1], Josephine Tang[1], Ruth Alexander[1] and Ravindra Sharma[1*]

[1]Department of Pathobiology, School of Veterinary Medicine, St. George's University, Grenada, West Indies.

Authors' contributions

This work was carried out in collaboration between all authors. Author RMK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KT and JT managed the trapping of rats and collection of blood after opening the rats. Author RA carried out laboratory analysis of blood samples. Author RS managed the literature searches and completed the final manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/41535
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Marcia Ratner, Boston University School of Medicine, USA.
(2) U. K. Amah, Nnamdi Azikiwe University, Nigeria.
(3) Helen Udujih, Imo State University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/24690

Short Research Article

Received 10th March 2018
Accepted 16th May 2018
Published 19th May 2018

ABSTRACT

Aim: Brown rats (*R. norvegicus*), a natural intermediate host for *Cysticercus fasciolaris*, are widely distributed in Grenada. *C. fasciolaris* causes cysts in a number of organs, but with greater frequency in the liver. The purpose of this study was to investigate the alterations of serum chemistry particularly serum proteins and activity of liver enzymes associated with liver cysts in brown rats infected with *C. fasciolaris,* as well as to assess their performance in the diagnosis of *Cysticercus fasciolaris*.
Study Design: In this cross sectional study, 170 brown rats were anesthetized and blood was obtained directly from the heart and collected in red-top tubes. The rats were then euthanized, and dichotomously classified as positive or negative for *Cysticercus fasciolaris* based on the presence or absence of *Cysticercus fasciolaris*-associated cysts, grossly and/or histopathologically.

**Corresponding author: E-mail: rsharma@sgu.edu;*

Methodology: For both groups of rats (positive or negative), the concentrations of each specific protein (g/dL), selected liver and pancreatic enzymes (U/L), metabolic waste products (mg/dL) and electrolytes (mg/dL) were measured using Vet Test (IDEXX, USA). Cut-off points for test values of liver enzymes were set at 2-fold or greater than upper reference limit. For all other analytes, test values that were outside the reference interval were considered to be diagnostically relevant. In order to determine the diagnostic performance and agreement between liver enzymes and *Cysticercus fasciolaris*-associated liver cysts, predictive values and Cohen's kappa statistics, respectively were calculated.

Results: The activity of AST significant increased among the infected brown rat population. The sensitivity and specificity of AST in detecting *Cysticercus fasciolaris* in brown rats was 76% and 59%, respectively. The positive and negative predictive values of AST were (80%) and (35%) respectively. Based on Cohen's kappa, AST showed good agreement to gross/histopathology [0.4, 95% CI 0.16 – 0.53, SE_{kappa} 0.42] in the detection of *Cysticercus fasciolaris*-associated liver cysts.

Conclusion: AST is the most reliable enzyme in detecting *Cysticercus fasciolaris* in brown rats, and thus *C. fasciolaris* should be included among the differential diagnoses whenever increased serum activity of AST are observed in brown rats.

Keywords: Grenada, Aspartate aminotransferase, Cysticercus fasciolaris, kappa, brown rats, predictive values.

1. INTRODUCTION

Cats are the definitive hosts of *Taenia taeniaeformis*, but the metacestode, *Cysticercus fasciolaris*, is found in a number of intermediate hosts that include rodents, birds and humans [1]. In Grenada, *Cysticercus fasciolaris* has been reported in brown rats (*Rattus norvegicus*) with a high prevalence and diagnostic frequency [2,3].

There are contradicting reports regarding the clinical presentation of infected intermediate hosts. Some studies have documented that infection of brown rats with *Cysticercus fasciolaris* is asymptomatic [4,5], but other studies have reported abdominal distension, lethargy and anorexia among populations of rats infected with the larval stage. Generally, these clinical signs are non-specific, and thought to be induced or secondary complications [6,7].

On gross and histopathology, the most distinct lesion associated with *Cysticercus fasciolaris* in infected intermediate hosts is the presence of cystic lesions in liver, and less frequently kidney, lungs [2]. Intermittently, hyperplastic gastropathy and enteropathy have been reported in the intermediate host *Cysticercus fasciolaris* [8,9,10].

In most species of animals, hepatic lesions are known to cause alterations in serum activities of liver enzymes and protein profiles [11,12,13,14]. Accordingly, serum chemistry has been used as a low-cost and low-risk screening tool to assess hepatocellular disease in livestock, companion and wild animals. However, we are not aware of any reports on the effect of *Cysticercus fasciolaris* on serum proteins and activity of liver enzymes in populations of naturally-infected intermediate hosts. Thus, the purpose of this study is to determine if the presence of *Cysticercus fasciolaris*-associated liver cysts in *Rattus norvegicus* causes remarkable alterations in the activity of liver enzymes or other selected serum chemistry parameters, and the diagnostic-suitability of these chemistry alterations in infected brown rats.

2. MATERIASLS AND METHODS

2.1 Collection of Rats

The study was approved by the Institutional Animal Care and Use Committee (IACUC # 16009-R) of the St. George's University Grenada. Grenada is the southernmost country in the Caribbean Sea, and its tropical climate consisting of low hills, small trees and shrubs, is most suitable for the existence of brown rats. One hundred and seventy live brown rats were collected between May 1 and July, 2017, using live traps (45 cm l x 15 cm w x 15 cm h) using cheese or various local fruits as bait. Attempts were made to trap the rats from and near the residential buildings. Traps were placed in the evening and visited the morning of the following day. Traps with rats were covered with black cloth and transported to the necropsy laboratory of the School of Veterinary Medicine, St. George's University.

2.2 Collection of Blood and Analysis of Samples

Rats were anesthetized by means of isoflurane in oxygen using a portable veterinary anesthesia machine; isoflurane vaporizer (VET CE, Avante Health Solutions, USA). The anesthetized rats were examined physically for their health and weighed. Blood was collected from the heart through the thoracic wall and placed in plain (red-top) blood tubes. Following exsanguination, the abdominal cavity of rats was opened using a surgical blade and a pair of forceps. Liver, lung, kidney and abdominal cavity were examined and recorded for gross lesions of *Cysticercus fasciolaris.* The parasites were removed from the cysts and examined. Those tissues with gross lesions were fixed in 10% neutral buffered formalin, processed for paraffin embedding, sectioned at 4 µm thickness, stained with hematoxylin and eosin and examined under the light microscope.

Sera were separated from the blood by centrifugation at 1500 g for 15 minutes at room temperature and analyzed thereafter. Hemolyzed samples were excluded from study. The concentrations of protein (g/dL), globulin (g/dL), albumin (g/dL), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), amylase, cholesterol (mg/dL), serum urea nitrogen (SUN), creatinine, calcium and phosphate in serum were measured using VetTest (IDEXX, USA).

2.3 Statistical Methods

Brown rats were dichotomously classified as positive or negative for *Cysticercus fasciolaris* on the basis of gross and histological presence or absence of liver cysts, respectively. The student's t-test in PHStat, Excel was used to determine the differences in the mean concentrations of each serum chemistry analytes between *Cysticercus fasciolaris*-positive rats and *Cysticercus fasciolaris*-negative rats. These were further stratified by gender of the brown rats.

This was followed by comparing each rat's test value with the reference interval (RI) established for that parameter. Cut-off points for test values of enzymes were set at 2-fold or greater than upper reference limit (URL). Thus, only the following test values: ALP ≥ 604U/L; GGT ≥ 12 U/L; ALT ≥ 122U/L; AST ≥ 222U/L; AMYL ≥ 4592U/L were considered diagnostically-useful. For all other analytes, test values that were below reference interval (bri) or above reference interval (ari) were considered to be diagnostically-relevant. The relative proportions of rats with abnormal values (ari or bri) for each parameter were compared between *Cysticercus fasciolaris* positive and *Cysticercus fasciolaris* negative rats using the chi-square test in EPIINFO at a level of significance, α=0.05.

Using gross and histological lesions of *Cysticercus fasciolaris*-associated liver cysts as the gold standard diagnostic protocol, the performance of alterations in serum chemistry, in characterizing these lesions was assessed in terms of sensitivity, specificity, positive and negative predictive values. Given that predictive values are normally affected by prevalence of a disease, Cohen's kappa was also used to further assess the percent agreement between serum chemistry and gross/histopathology in diagnosing *Cysticercus fasciolaris* in rats.

3. RESULTS

Clinically, all rats appeared to be in good health upon presentation to the Necropsy Laboratory. Results are presented in Table 1. Of the 4 liver enzymes, high activities were only observed in AST and ALT, but only AST was diagnostically-relevant in differentiating *Cysticercus fasciolaris*-positive and *Cysticercus fasciolaris*-negative rats. Additionally, AST showed better sensitivity (76%) than ALT (11%), but its specificity was lower (59%) compared to ALT (95%). Accordingly, AST had a better positive predictive value (PPV) (80%) and negative predictive value (NPV) (35%) than ALT (PPV=50%; NPV=28%). Likewise based on Cohen's kappa, AST showed better agreement [0.4, 95% CI 0.16 – 0.53, SE_{kappa} 0.42] to gross/histopathology when compared with ALT [0.04, 95% CI -0.02 – 0.09, SE_{kappa} 0.094].

Based on the gross and histologic evaluation, the prevalence of *Cysticercus fasciolaris* in brown rats in Grenada was 67% (115/170), while its prevalence was established to be 45% (57/103) using AST and 7.3% (10/136) using ALT.

There were no statistically significant differences in the mean serum chemistry when *Cysticercus fasciolaris*-positive wild rats were compared with *Cysticercus fasciolaris*-negative wild rats. Further, stratification of the wild rats by gender did not also yield significant differences in mean concentrations of the same serum chemistry parameters (results not shown).

Table 1. Number of rats with alterations in the concentration of selected serum chemistry points stratified by *Cysticercus fasciolaris* status

Parameter	Units	RI	cut-off	# of rats	
				TT positive	TT negative
Protein	g/dL	5.3 - 6.9	< 5.3	4	3
			5.3 - 6.9	37	41
			> 6.9	34	14
Albumin	g/dL	3.8 - 4.8	< 2.7	9	4
			2.8 - 4.8	47	49
			> 4.8	14	10
Globulin	g/ dL	1.5 - 2.8	< 2.8	2	1
			1.5 - 2.8	44	45
			> 2.9	34	17
ALP	U/L	16 - 302	≤ 603	87	35
			≥ 604	6	1
GGT	U/L	1 - 6	≤ 11	90	37
			≥ 12	0	2
ALT	U/L	20 - 61	≤ 121	85	39
			≥ 122	10	2
AST	U/L	39 - 111	≤ 221	15	24
			≥ 222	47	17
AMYL	U/L	326 - 2246	≤ 4491	72	53
			≥ 4492	0	0
Cholesterol	mg/dL	20 - 92	≤ 20	2	5
			20 - 92	90	25
			> 92	6	5
SUN	mg/dL	9 - 21	≤ 21	77	34
			> 21	22	6
Creatinine	mg/dL	0.1 - 0.6	≤ 0.6	86	32
			> 0.6	2	1
Calcium	mg/dL	5.3 - 11.6	≤ 5.3	2	3
			5.3 - 11.6	58	36
			> 11.7	0	0
Phosphate	mg/dL	5.8 - 11.2	≤ 5.8	1	0
			5.8 - 11.2	116	53
			> 11.2	0	0

Protein (X^2=6.4, df =1p< 0.01)

4. DISCUSSION

It is remarkable that such a high prevalence of *Cysticercus fasciolaris* in brown rats was not supported by the presence of clinical signs. This is symbolic of a well-established host-parasite relationship that ensures survival and development of *Cysticercus fasciolaris* without harming brown rat [6].

It has been suggested that under natural conditions, the primary infection of rodents with *Cysticercus fasciolaris* leads to a strong immunity, and thus, restricts the development of additional cysts in the liver [15]. The increased activity of mostly cytosolic (AST and ALT), but not membrane bound (ALP and GGT) liver enzymes indicates that the cysts induced by *Cysticercus fasciolaris* lead to restricted hepatocellular injury as opposed to induction [16]. The elevated serum activity of AST can be used to characterize wild rats with a high probability of *Cysticercus fasciolaris* than ALT, probably because of the longer half-life of AST. Given this performance characteristic, AST is the most promising serum-based test for diagnosing *Cysticercus fasciolaris* in infected brown rats. Similar performance was obtained for AST in the detection of hepatic lipidosis in cattle [14]. The increased activity of AST can occur as a result of myocyte injury [17,18] and damage to erythrocytes [19]. We did not include hemolyzed samples in our study, and thus controlling for erythrocytes being the source of AST. However, we did not measure the activity of creatinine kinase (CK), which originates in muscle tissue.

Thus further studies that include the measurement of CK in wild rats with *Cysticercus fasciolaris* are encouraged.

Hyperproteinemia due to a selective hyperglobulinemia is consistent with an inflammatory response to the collagen capsule surrounding *Cysticercus fasciolaris* [20]. *Cysticercus fasciolaris* infection in brown rats has no significant alteration in the concentration of electrolytes, cholesterol, amylase and serum urea nitrogen and creatinine.

5. CONCLUSION

C. fasciolaris should be included among the differential diagnoses if increased serum activity of AST is observed in brown rats.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

ACKNOWLEDGEMENT

Authors thank St. George's University Grenada for fund for the research project through One Health Research Initiative (OHRI 06-14-10) grant.

REFERENCES

1. Rodríguez-Vivas RI, Panti-May JA, Parada-Lopez J, Hernández-Betancourt SF, Ruiz-Piña HA. The occurrence of the larval cestode *Cysticercus fasciolaris* in rodent populations from the Cuxtal ecological reserve, Yucatan, Mexico. Journal of Helminthology. 2011;85:458-461.
2. Coomansingh C, Pinckney RD, Bhaiyat MI, Chikweto A, Bitner S, Baffa A, Sharma R. Prevalence of endoparasites in wild rats in Grenada. West Indian Veterinary Journal. 2009;9:17-21.
3. Sharma R, Keshaw T, Kristen B, Elan A, Andrea M, Reneka G, Yvette S, Veronica Mapp-A, Claude D. *Cysticercus fasciolaris* in brown rats (*Rattus norvegicus*) in Grenada, West Indies. Journal of Parasitology Research. 2017;4. Article ID1723406
4. Kataranovski M, Zolotarevski L, Belij S, Mirkov I, Stošić J, Popov A, and Kataranovski D. First record of *Calodium hepaticum* and *Taenia taeniaeformis* liver infection in wild Norway rats (*Rattus norvegicus*) in Serbia. Archives of Biological Sciences. 2010;62:431-440.
5. Claveria FG, Causapin J, de Guzman MA, Toledo MG, and Salibay C. Parasite biodiversity in *Rattus* spp. caught in wet markets. South Asian J Trop Public Health. 2005;36:146–148.
6. Irizarry-Rovira AR, Wolf A, Bolek M. Taenia taeniaeformis-induced metastatic hepatic sarcoma in a pet rat (*Rattus norvegicus*). Journal of Exotic Pet Medicine. 2007;16:45-48.
7. Karim A. Scanning electron microscopy and histological morphology of *Cysticercus fasciolaris*, which induced fibrosarcomas in laboratory rats. Annals of Microscopy. 2010;10:44-8.
8. Cook RW, Williams JF, Lichtenberger LM. Hyperplastic gastropathy in the rat due to *Taenia taeniaeformis* infection: Parabiotic transfer and hypergastrinemia. Gastro-enterology. 1981;80:728-734.
9. Cook RW, Williams JF. Pathology of *Taenia taeniaeformis* infection in the rat: Gastrointestinal changes. Journal of Comparative Pathology. 1981;91:205-217.
10. Konno K, Abella JA, Oku Y, Nonaka N, Kamiya M. Histopathology and physiopathology of gastric mucous hyperplasia in rats heavily infected with *Taenia taeniaeformis*. Journal of Veterinary Medical Science. 1999;61:317-324.
11. Russell KE, Roussel AJ. Evaluation of the ruminant serum chemistry profile. Veterinary Clinics: Food Animal Practice. 2007;23:403-426.
12. Boyd JW. Serum enzymes in the diagnosis of disease in man and animals. Journal of Comparative Pathology. 1988;98(4):381-404.
13. Ramaiah SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food and chemical toxicology. 2007;45:1551-1557.
14. Cebra CK, Garry FB, Getzy DM, Fettman MJ. Hepatic lipidosis in anorectic, lactating Holstein cattle: A retrospective study of serum biochemical abnormalities. Journal

of Veterinary Internal Medicine. 1997;11: 231-237.

15. Al-Salihi KA, Sheikh A. and Saied H. High prevalence *of Strobilocercus fasciolaris* infection associated with gastroentropathy and hepatic fibrosarcoma between laboratory rats in experimental groups. African Journal of Animal and Biomedical Sciences. 2009;4:6–10.
16. Kim W, Flamm SL, Di Bisceglie AM, Bodenheimer HC. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology. 2008;47: 1363-1370.
17. Valberg S, Jonsson L, Lindholm A, Holmgren N. Muscle histopathology and plasma aspartate aminotransferase, creatine kinase and myoglobin changes with exercise in horses with recurrent exertional rhabdomyolysis. Equine Veterinary Journal. 1993;25:11-16.
18. O'Neill SL, Feldman BF. Hemolysis as a factor in clinical chemistry and hematology of the dog. Veterinary Clinical Pathology. 1989;18:58-68.
19. Rahul A Nathwani, Shireen P, Telfer B. Raynolds, Neil K. Serum alanine aminotransferase in skeletal muscle diseases. Hepatology. 2005;41(2):1-7. Available:https://doi.org/10.1002/hep.20548
20 Singla LD, Singla N, Parshad VR, Sandhu BS, Singh J. Occurrence and pathomorphological observations of *Cysticercus fasciolaris* in lesser bandicoot rats in India. Aciar Monograph series, 2003;96:57-60.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24690