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RESEARCH ARTICLE

First Comparative Biochemical Profile Analysis of Cystic Fluids of *Taenia hydatigena* and *Echinococcus granulosus* Obtained from Slaughtered Sheep and Goats

Mughees Aizaz Alvi[1], Li Li[1], Sami Ullah Khan Bahadur[2], Muhammad Saqib[3], John Asekhaen Ohiolei[1], Rana Muhammad Athar Ali[3], Imaad Rashid[3], Khurram Ashfaq[3], Asif Ali Butt[4], Muhammad Zaeem Abbas[5], Muhammad Haleem Tayyab[3], Amjad Islam Aqib[6], Warda Qamar[7], Ali Hassan[3], Anum Aizaz Alvi[5], Azhar Aslam[8], Bao-Quan Fu[1], Hong-Bin Yan[1*] and Wan-Zhong Jia[1*]

[1]State Key Laboratory of Veterinary Etiological Biology, National Animal Echinococcosis Para-Reference Laboratory, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, People's Republic of China; [2]The Fleming Fund Country Grant Pakistan, DAI, Islamabad, 44000; [3]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan; [4]Riphah University, Faisalabad, Pakistan; [5]Independent Researcher, Faisalabad, Punjab, Pakistan; [6]Department of Medicine, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan; [7]Department of Parasitology, University of Agriculture, Faisalabad, Pakistan; [8]Department of Microbiology, The Islamia University of Bahawalpur, Pakistan
*Corresponding author: jiawanzhong@caas.cn; yanhongbin@caas.cn

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ABSTRACT

The organic and inorganic constituents present in cystic fluid play a crucial role in the physiology, metabolism and immune responses mediated by taeniids. We designed a comparative study on *T. hydatigena* and *E. granulosus* cysts based on biochemical profile. In the present study, we aspirated fluid from *Taenia hydatigena* (n=30) and *Echinococcus granulosus* (n=30) cysts recovered from slaughtered sheep and goats in the central abattoir of Faisalabad, Pakistan. The fluid obtained from each cyst was subjected to biochemical analysis to estimate the levels of liver enzymes, electrolytes and selected biomolecules (proteins, glucose, cholesterol, urea, and creatinine). Thereafter, the student's t-test was used to determine significant differences between constituents of the cystic fluids obtained from both type of cysts. The difference was considered statistically significant at $P<0.05$. The levels of total protein and globulin differed significantly ($P<0.05$) and were found to be higher in *T. hydatigena* cystic fluid. A non-significant difference was observed for albumin. Similarly, higher concentrations of electrolytes (sodium, chloride and magnesium) were observed in *T. hydatigena* cystic fluid with statistically significant difference ($P<0.05$). Concerning liver enzymes, high levels ($p>0.05$) of ALP and LDH were observed in *T. hydatigena* and *E. granulosus* cystic fluid had significantly higher ($P<0.05$) level of AST. The levels of cholesterol, urea and creatinine were higher in *E. granulosus*. In contrast, glucose was higher in *T. hydatigena*. A significant difference ($P<0.05$) was observed for these four biomolecules. Overall, these results suggest high variations of investigated biomolecules, liver enzymes, and electrolytes composition between cystic fluids of *E. granulosus* and *T. hydatigena*. Further studies are now warranted to set the standards for the differential diagnosis of cysticercosis and cystic echinococcosis based on cystic fluid contents.

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INTRODUCTION

Members of the family Taeniidae infect carnivores as their definitive hosts and several other animal species including domesticated animals act as intermediate hosts (Irshadullah and Rani, 2011). *Echinococcus granulosus* and *Taenia hydatigena* are among the important members of this family and infest visceral organs of sheep, goat and

cattle leading to the development of diseases called cysticercosis and hydatidosis, respectively (Abdel-Baki *et al*., 2018; Mokhtaria *et al*., 2018). Both parasites have serious setbacks on livestock production with *E. granulosus* demonstrating stern zoonotic impacts (Yakhchali *et al*., 2017; Assana *et al*., 2019).

The eggs released in faeces of dogs and other definitive hosts contaminate the herbage and water channels which lead to the transmission of infective stages to the intermediate host through ingestion (Elham *et al*., 2014; Tsotetsi-Khambule *et al*., 2017). Predilection sites of both the parasites in the intermediate hosts are diverse as *T. hydatigena* may find its seat in liver, omentum, spleen, heart and kidney (Assana *et al*., 2019) while *E. granulosus* forms the cysts primarily in the liver and in a few cases lungs and brain can be the tissues for cyst development (Conceicao *et al*., 2017).

The fluid present within the cysts is clean and clear containing secretions from both the parasite and host. This fluid is important for the development and growth of cysts that eventually give rise to the adult worm in the definitive hosts (Zhang *et al*., 2016). There are different organic and inorganic components present within the cystic fluid and differ quantitatively based on the cyst location and host origin (Radfar *et al*., 2012). These constituents also differ according to the strains or haplotypes playing an imperative role in the growth, metabolism, physiology and immunology of the cyst (Rahdar *et al*., 2008).

Although many studies were carried out to understand the composition of cystic fluid, biochemical constitution of cystic fluid differs with parasitic species. It was found that no attention was paid on comparative analysis of composition of cystic fluid between *E. granulosus* and *T. hydatigena*. Thus, it is very important to conduct a comparative study to identify variations in composition of cystic fluid between species which can further be used in pathological and immunological studies for species diagnosis. To close this research gap, the current study was designed to report the differences in chemical and biochemical profiles of *T. hydatigena* and *E. granulosus* cystic fluids.

MATERIALS AND METHODS

Study area and sampling: Cyst samples were collected from abattoirs located in Faisalabad district in Punjab province of Pakistan. The visceral organs (liver, lungs, and kidney) of the slaughtered sheep and goats were checked thoroughly for the presence of *T. hydatigena* or *E. granulosus* cysts. Inspection of the organs and confirmation of *Taenia* cysts was done using the guidelines of Manual (Diagnostic Tests and Vaccines for Terrestrial Animals) (OIE, 2019) whereas *Echinococcus* cysts were recognized as per recommendations of WHO/OIE Manual on *Echinococcosis* in Humans and Animals (Eckert *et al*., 2001). *Echinococcus granulosus* cysts were collected from liver and lungs while *T. hydatigena* cysts were recovered from liver. The cysts were carefully removed, placed in sterile plastic containers, and serum samples were collected into gel-clot activator serum vials (Improvacuter, Hamburg, Germany) and transported to the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. A total of 60 cysts, 30 from each tapeworm species were collected and depending on the cyst size, approximately 8 ml of cystic fluid was aspirated using a sterile Gosselin™ pipette into sterile tubes. Tubes were centrifuged at 2000 rpm at 4°C for 15 min and the supernatant was removed and stored at -40°C until used (Li *et al*., 2013). Sera were collected after centrifugation (4,000 rpm for 15 minutes) in cryovials and kept at -20ºC until needed (Afridi *et al*., 2017).

DNA extraction, amplification and sequencing: DNA extraction from each cyst was carried out using using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). PCR was performed in reaction mixture of 25 µl volume consisted of 12.5 µl Premix (Takara Bio, Kusatsu, Japan), 10 pmol reverse and forward primers, 0.5 µl genomic DNA, and RNAse free, ultra-pure PCR water up to final volume. As negative controls, RNAse-free water was used instead of DNA in each group of PCR reactions. Previously developed primer pair i.e. JB3 (5′TTTTTTGGGCATCCTGGTTTAT3′) and JB4.5 (5′-TAAAGAAAGAACATAATGAAAATG-3′) were used for amplification of part of the *cox*1 gene (Bowles *et al*., 1992). Initial denaturation at 94°C for 5 minutes was followed by 35 cycles of denaturation (30 s at 94°C), annealing (45 s at 50°C) and extension (35 s at 72°C). A final extension at 72°C for 10 minutes was carried out. Five microliters of amplicon from PCR products were visualized in each well of 1.5% agrose gel stained with GelRed™. A 2000-bp ladder was used to estimate the length of amplicon. Then amplicons were sent for sequencing and BLAST analysis was done and, in this way, molecular confirmation of the cysts was carried out.

Biochemical testing: The levels of calcium, magnesium, and chloride in stored supernatant were measured as described previously by Kaneko *et al*. (1997) while the concentrations of sodium and potassium were determined as performed by Mozaffari *et al*. (2011). Alkaline phosphatase (ALP) and alanine transaminase (ALT) levels in serum were measured spectrophotometrically according to the method by Burger *et al*. (2005). Ultra-violet method was employed to determine the level of aspartate aminotransferase (AST) (Bergmeyer *et al*., 1986). Lactate dehydrogenase (LDH) and protein concentrations were measured through colorimetric method (Marutsova and Binev, 2020). The methodology adopted to determine the levels of glucose, urea, triglycerides and cholesterol was based on enzymatic colorimetric method as described by Omidi *et al*. (2018). For quantification of creatinine, Jaffe method was used (Omidi *et al*. 2018).

Statistical analysis: The means and standard error of means of the biochemical constituents were calculated using IBM SPSS version 21 (IBM Corp. Armonk, NY). The student's t-test was applied to determine significant differences between different constituents of cystic fluids obtained from *E. granulosus* and *T. hydatigena*. The difference was considered statistically significant with values of $P<0.05$. GraphPad Prism Software was used for the representation of the results in the form of graphs.

RESULTS AND DISCUSSION

All collected isolates of *Echinococcus granulosus* and *Taenia hydatigena* were found positive as a segment of *cox*1 gene was successfully amplified as shown in Fig. 1.

Apart from infecting multiple animal species, taeniasis and echinococcosis have zoonotic importance as well i.e. transmitted from animals to humans (Eckert and Deplazes, 2004; Scandrett *et al*., 2009; Kumar *et al*., 2016). A definitive diagnosis is an essential element for the control of these diseases. To diagnose and/or differentiate cysticercosis (*T. hydatigena*) and echinococcosis / hydatidosis (*E. granulosus*), biochemical analysis of fluids aspirated from cysts can serve the purpose, as these biochemical contents play a definitive role in the physiology, metabolism and immunology of the disease (Chowdhury and Singh, 2009). As compared with serum level, a significant difference in values of albumin and globulin, glucose, urea, creatinine, cholesterol, electrolytes, and liver enzymes of *Echinococcus* cyst fluid of infected sheep and goat was observed and represented graphically in Fig. 2 (2a, 2b, 2c, 2d and 2e) and similar changes were also recorded in case of *Taenia* cyst fluid as shown in Fig. 3 (3a, 3b, 3c, 3d and 3e).

This study was conducted to assess the biochemical composition of hydatid fluid and cysticercosis cystic fluid from infected animals. Differentiation of biochemical contents of cysts in the case of cysticercosis and echinococcosis has a role in the differential diagnosis of both conditions. The results demonstrate a significant ($P<0.05$) difference for many biochemical components of the cysts when compared with the serum reference range in infected animals which indicates differential points for these conditions.

Proteins: All proteins i.e. total proteins, albumin and globulin were considerably lower in cystic fluids from *T. hydatigena* and *E. granulosus* metacestodes than the normal proteins levels in the serum of intermediate hosts (Table 1). *Taenia* cyst fluid had 0.95±0.10 g/dL, 0.44±0.07 g/dL and 0.50±0.03 g/dL while *E. granulosus* cystic fluid had 0.75±0.03 g/dL, 0.32±0.02 g/dL, and 0.43±0.02 g/dL of total proteins, albumin and globulin, respectively (Fig. 4). There was a significant ($P<0.05$) difference in the levels of total proteins and globulins between *T. hydatigena* and *E. granulosus* cysts but no statistical difference ($P>0.05$) was observed for albumin concentration in both cyst aspirates.

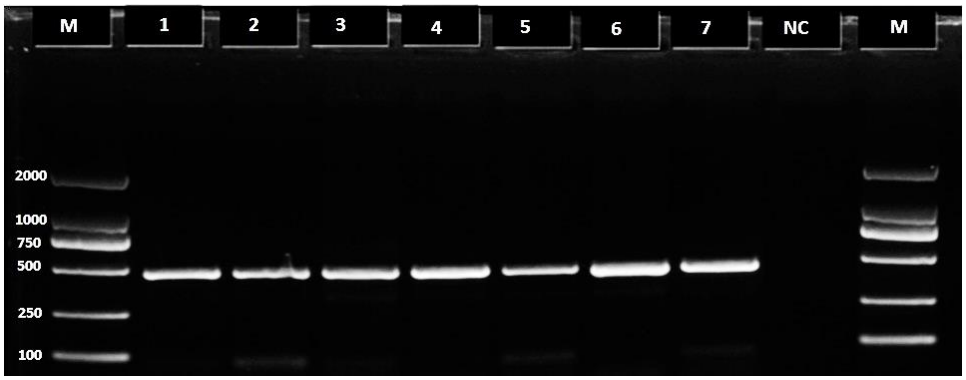

Fig. 1: Image of 1.5% agarose gel viewed through trans-illuminator.

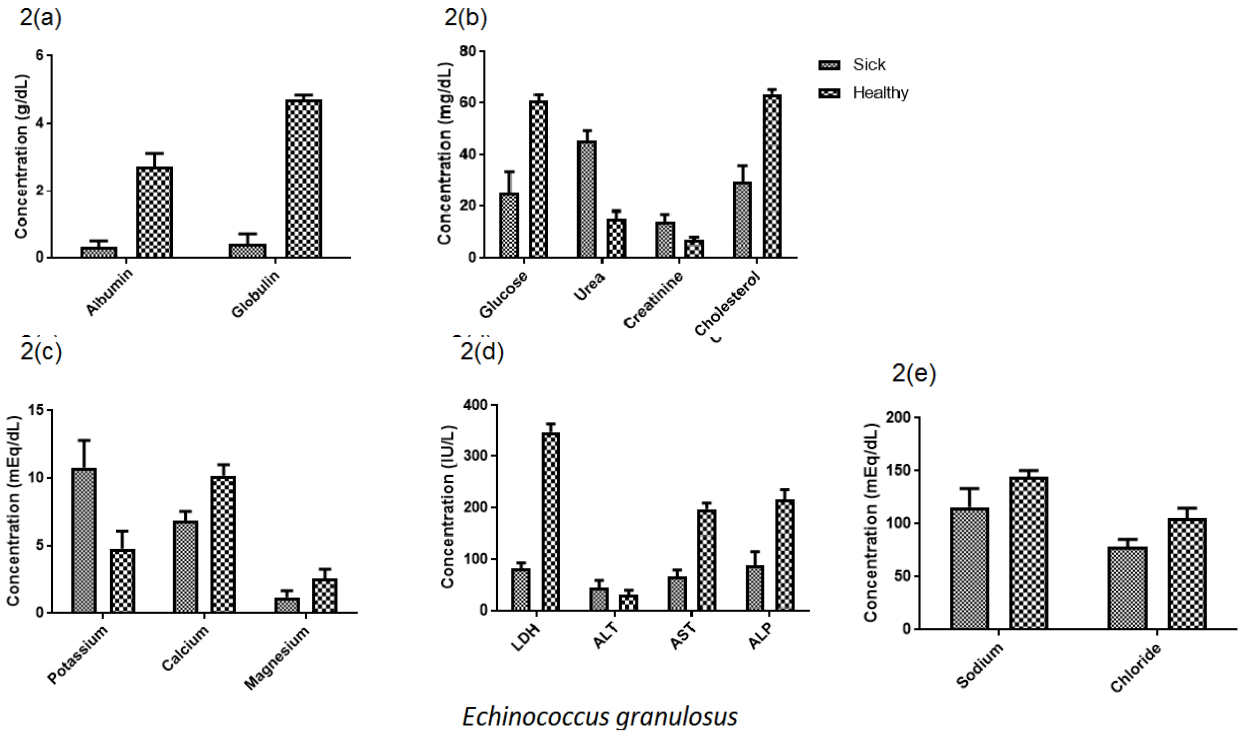

Fig. 2: Comparison of biochemical changes of *Echinococcus granulosus* cyst fluid with serum of healthy animal.

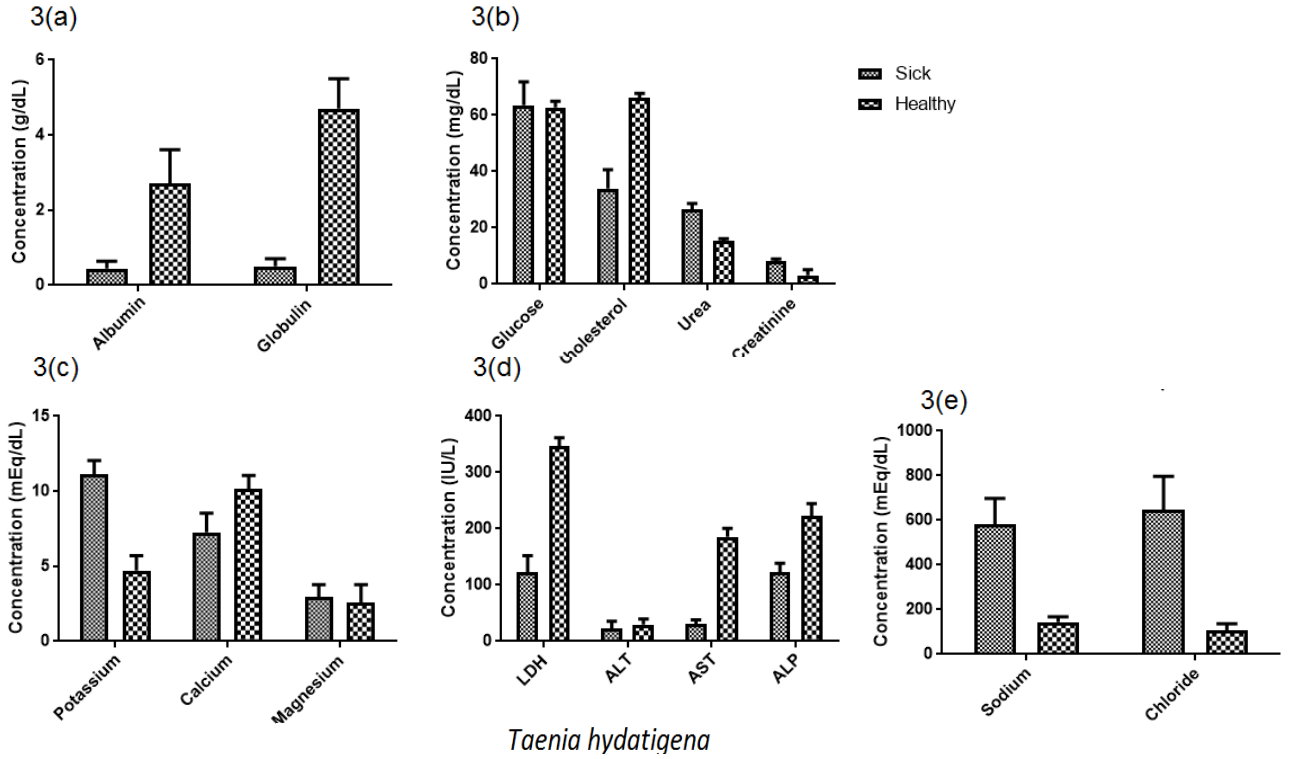

Fig. 3: Comparison of biochemical changes of *Taenia hydatigena* cyst fluid with serum of healthy animal.

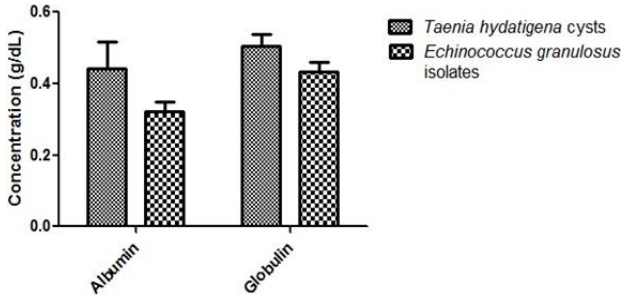

Fig. 4: Levels of albumin and globulin in *Taenia hydatigena* and *Echinococcus granulosus* cyst fluid.

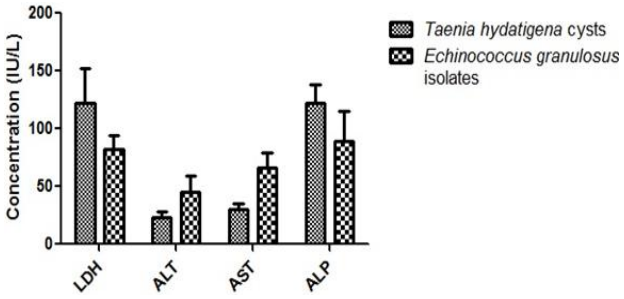

Fig. 5: Levels of LDH, ALT, AST and ALP in *Taenia hydatigena* and *Echinococcus granulosus* cystic fluid.

Although all proteins i.e., total proteins, albumin and globulins were quite lower in both types of cysts than the respective serum levels, the statistical difference suggests that total proteins and globulins could be potential differential entities for both conditions. Previous studies by Shaafie *et al*. (1999) and Latif *et al*. (2020) also had shown similar/lower levels of proteins in *Echinococcus* cyst fluid than levels found in the serum. In hydatidosis, cyst fluid had double the level of globulins than albumin (Frayha and Haddad, 1980). A similar pattern for cysticercosis was also found in this study. Higher level of protein (globulin) describes its significance in growth related anabolic and catabolic activities (Dow *et al*., 1996). According to Mohammed (2020), the protein level in *Coenurus cerebralis* fluid was comparable with protein serum level but considerably higher than our findings.

Liver enzymes: The level of liver enzymes, including ALT, AST, ALP and LDH in cystic fluid of *T. hydatigena* with concentration (Mean±SEM) of 22.68±5.91 IU/L, 30.10±5.46 IU/L, 122.10±16.37 IU/L and 122.47±29.66 IU/L, respectively was considerably lower than the serum reference range (Fig. 5).

The pattern of concentration enzymes in the fluid of *Echinococcus* cyst was different from that of *Taenia* e.g. LDH (82.10±11.82 IU/L; Mean±SEM) was lower and ALT (45±14.29 IU/L; Mean±SEM) was higher than serum levels while the levels of AST (66.52±12.99 IU/L; Mean±SEM) and ALP (89.10±26.44 IU/L; Mean±SEM) were within normal serum reference range (Table 2). With these concentrations, fluids collected from cysts of small ruminants infected with *T. hydatigena* and *E. granulosus* had significant ($P<0.05$) difference in the levels of AST while no statistical difference was observed in the levels of LDH, ALT and ALP.

With regard to the comparison of levels of enzymes in cystic fluids of both the conditions, an enormously significant ($P<0.05$) difference was found between fluids aspirated from cysticercosis and hydatid cysts. We found that in case of echinococcosis, ALT (65.44±16.44 IU/L) and AST level (107±14.73 IU/L) were higher and within serum reference range, but Abdelrahman *et al*. (2011) found significantly higher levels of both the enzymes. The level of ALT, AST, ALP and LDH were lower in cystic fluid of *T. hydatigena* and this was in coherence with the findings of Nath *et al*. (2010).

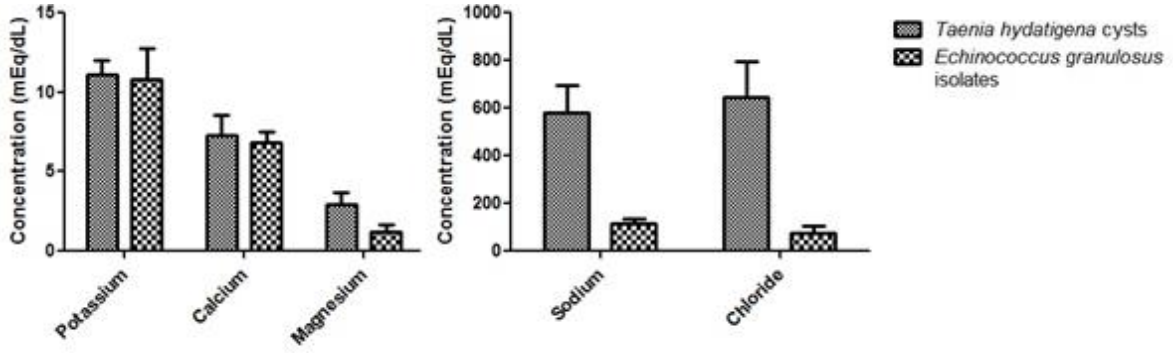

Fig. 6: Levels of glucose, urea and cholesterol in *Taenia hydatigena* and *Echinococcus granulosus* cystic fluid.

Table 1: Levels of protein in fluids obtained from *Taenia hydatigena* and *Echinococcus granulosus* cysts.

Component	*Taenia*	*Echinococcus*	*Taenia*	*Echinococcus*	p-value	Serum Reference Range
	Mean±SEM		Range			
Total proteins (g/dL)	0.95±0.10	0.75±0.03	0.2-1.6	0.5-1.1	<0.05	6.1-7.5
Albumin (g/dL)	0.44±0.07	0.32±0.02	0-1	0.1-0.5	NS	2.4-3
Globulin (g/dL)	0.50±0.03	0.43±0.02	0.2-0.7	0.2-0.8	<0.05	3.5-5.7

Table 2: Levels of different enzymes in *Taenia hydatigena* and *Echinococcus granulosus* cystic fluids.

Component	*Taenia*	*Echinococcus*	*Taenia*	*Echinococcus*	p-value	Serum Reference Range
	Mean±SEM		Range			
Alanine aminotransferase (IU/L)	22.68±5.91	45±14.29	1-115	2-200	NS	26-34
Aspartate aminotransferase (IU/L)	30.10±5.46	66.52±12.99	2-83	8-174	<0.05	60-280
Alkaline phosphatase (IU/L)	122.10±16.37	89.10±26.44	3-265	5-488	NS	68-387
Lactate dehydrogenase (IU/L)	122.47±29.66	82.10±11.82	14-445	25-208	NS	238-440

Table 3: Levels of different electrolytes in *Taenia hydatigena* and *Echinococcus granulosus* cystic fluids.

Component	*Taenia*	*Echinococcus*	*Taenia*	*Echinococcus*	p-value	Serum Reference Range
	Mean±SEM		Range			
Sodium (mEq/L)	580.79±117.20	115.78±17.56	105.5-1305	24.8-294	<0.05	137-152
Chloride (mEq/L)	646.12±150.65	78.20±7.26	95.2-1792	27.3-137.6	<0.05	100-112
Potassium (mEq/L)	11.12±0.91	10.78±2.00	5.67-19.13	3.87-39.51	NS	3.8-5.7
Calcium (mEq/L)	7.26±1.26	6.83±0.71	0.9-14.1	2.2-14.4	NS	9.0-11.6
Magnesium (mEq/L)	2.96±0.20	1.17±0.22	1.3-4.4	0.1-4	<0.05	2.1-2.9

Table 4: Levels of different biomolecules in *Taenia hydatigena* and *Echinococcus granulosus* cystic fluids.

Component	*Taenia*	*Echinococcus*	*Taenia*	*Echinococcus*	p-value	Serum Reference Range
	Mean±SEM		Range			
Glucose (mg/dL)	63.52±8.29	25.26±8.03	15-125	5-154	<0.05	48-76
Cholesterol (mg/dL)	33.89±6.72	63.57±1.79	2-64	52-75	<0.05	52-76
Urea (mg/dL)	26.42±2.22	45.36±5.21	14-45	11-90	<0.05	8-20
Creatinine (mg/dL)	0.38±0.02	1.05±0.05	0.2-0.6	0.7-1.5	<0.05	0.7-1.5

Electrolytes: The sodium level in fluid from *T. hydatigena* and *E. granulosus* cysts collected from infected small ruminants was 580.79±117.20 mEq/L and 115.78±17.56 mEq/L, respectively (Fig. 6) and was considerably higher in the case of *T. hydatigena* and also when compared to normal serum range.

Chloride concentration had a similar pattern as that of sodium i.e. considerably higher (646.12±150.65 mEq/L) in *T. hydatigena* cysts and lower (78.20±7.26 mEq/L) in *E. granulosus* cysts (Table 3). Potassium level was higher than normal serum level in both cases with values (Mean±SEM) of 11.12±0.91 mEq/L and 10.78±2.00 mEq/L in *T. hydatigena* and *E. granulosus*, respectively. Calcium concentration was lower than serum level in both cyst fluids, and was 7.26±1.20 mEq/L and 6.83±0.71 mEq/L for *T. hydatigena* and *E. granulosus*, respectively. Magnesium level (2.96±0.20 mEq/L) in *T. hydatigena* cyst was within normal serum range but lower than the reference range in the case of *E. granulosus* (1.17±0.22 mEq/L). Between both cestodes, the concentration levels were significantly (P<0.05) different for sodium, chloride, and magnesium but not for potassium and calcium.

While comparing the level of sodium and chloride, there was significant difference with the higher and lower levels than respective serum levels in cysticercosis and echinococcosis, respectively but calcium had non-significant difference with the lower level than serum level in both conditions. According to Shaafie *et al*. (1999), sodium level in hydatid cystic fluid collected from cattle, sheep and goat was 83.50 mmol l^{-1}, 113.60 mmol l^{-1} and 133.66 mmol l^{-1}, respectively with lower concentration levels of calcium and a higher level of potassium compared to the serum concentration level. These findings correspond to our observations, but on the contrary, Radfar *et al*. (2012) who reported sodium levels in hydatid cyst fluid from liver and lung to be within normal serum range. However, pattern of potassium concentration reported by Radfar *et al*. (2012) supported our results as potassium, with non-significant difference, was higher than serum level in both conditions. For cysticercosis, potassium and sodium levels were very high which contradict to the finding of Mohammed (2020) who reported the levels of these electrolytes within the normal reference range. As far as magnesium concentration is

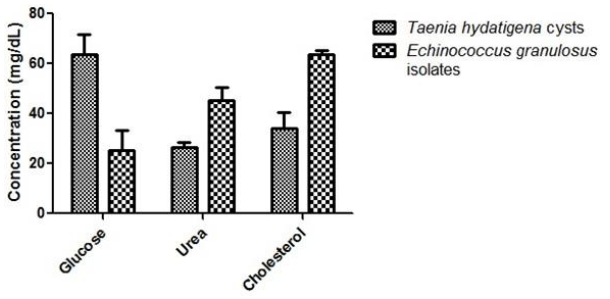

Fig. 7: Levels of glucose, urea and cholesterol in *Taenia hydatigena* and *Echinococcus granulosus* cystic fluid.

concerned, fluids of cysticercosis demonstrate normal magnesium level while the concentration in hydatid cyst was lower than serum range with a significant difference from cysticercosis. Calcium plays significant role in ATP synthesis while magnesium act as a cofactor in mediation different enzyme catalyzed reactions within hydatid cyst (Dow *et al*., 1996). Calcium is also involved in prevention of acidic environment and formation of calcareous objects within hydatid cyst. Calcium level may be found higher due to calcification and degenerative biochemical changes within hydatid cyst (Radfar *et al*., 2004).

Biomolecules: The glucose levels of fluids aspirated from both cysts were 63.52±8.29 g/dL and 25.26±8.03 g/dL for *T. hydatigena* and *E. granulosus*, respectively (Fig. 7, Table 4). The urea level 26.42±2.22 mg/dL in *Taenia* cyst was higher compared to what was observed in the serum range, but lower than that of *E. granulosus* 45.36±5.21 mg/dL. Cholesterol level was lower than serum concentration in both conditions. Creatinine concentration in both cyst fluids was found to be 0.38±0.02 g/dL and 1.0±0.05 g/dL, respectively. The concentration of each biomolecule in both cysts was statistically different ($P<0.05$).

Creatinine and cholesterol levels were lower in the cysts than serum levels in both cases. Cholesterol level varies with organ of involvement, and it is higher in liver cyst as compared with lungs which may be due to its different structural composition (Dow *et al*., 1996). According to Sheriff *et al*. (1989) cholesterol level increases with the degeneration of hydatid cyst. Shaafie *et al*. (1999) and Radfar *et al*. (2012) had also observed lower cholesterol and creatinine levels in hydatid cystic fluid than corresponding serum range. Cholesterol had also been found lower in hydatid cysts by Abdelrahman *et al*. (2011). Like hydatidosis, *Coenurus cerebralis* (*T. multiceps*) cyst fluid also possesses lower cholesterol levels (Mohammed, 2020) but is comparatively higher than our findings for *Cysticercus tenuicollis* (*T. hydatigena*). According to Dow *et al*. (1996), level of creatinine depicts ammonia catabolism and ATP production. The urea level in our study was higher than the serum reference range and this is supported by similar observations by Shaafie *et al*. (1999) and Radfar *et al*. (2012). Presence of urea in cystic parasite refers to the involvement of urea cycle necessary to decrease higher ammonia level through protein's building blocks metabolism (Dow *et al*., 1996). Lastly, the level of glucose in hydatid cyst was within normal serum reference range are demonstrated previously by other authors (Shaafie *et al*. 1999; Abdelrahman *et al*., 2011). Presence of glucose revealed that parasite cysts also generate energy through glycolysis pathways (Radfar *et al*., 2004).

Vuitton and Gottstein (2010) suggested that both host and parasite derived biomolecules constitute cyst fluid. Metabolism and biochemical parameters of the cyst fluid changes with intermediate host species (Radfar *et al*., 2004). These qualitative and quantitative differences in composition are probably due to intricate topographical strains, biochemical and physiological variance which can take place in different host animals around the world (Azami *et al*., 2013). Biochemical changes in cyst fluid composition might be the reason for different strain formation (Thompson, 1991; Shaafie *et al*., 1999). Moreover, shifts in metabolism of same species or strain of *E. granulosus* in different hosts take place that are necessary for survival in different environment (Thompson, 1991; Thompson and Lymbery, 1995) and same metabolic shifts and adaptability in variable environment may exist in case of *Taenia hydatigena*. Increasing and decreasing values of biochemical components provide some key information on defensive role of parasite cyst capsule to overcome the immunity induced by intermediate host. Therefore, biochemical studies are helpful in diagnosing species and strain variation. This parasitic species and strain based biomolecular characterization is of great concern in areas where various cyst causing parasitic species and their intermediate host reside.

Conclusions: We conclude that variation exists for the investigated biomolecules, enzymes, and electrolytes concentration of *E. granulosus* and *T. hydatigena* cyst fluids when compared to serum reference levels in infected animals. We recommend that considerable and detailed investigation be conducted to set the standards for the potential characteristics of these components in the diagnosis of taeniasis and echinococcosis.

Authors contribution: Conceptualization, MAA, LL, HBY and W-ZJ; methodology, MAA, W-ZJ, SUKB, MS, RMAA and AAA; formal analysis, MAA, MHT, WQ, MS, MZA, KA, AAB, and AIA; investigation, MAA, WQ, MZA, and MHT; funding acquisition, WZJ; data curation, MAA, JAO, IR, AA and AA; writing & original draft preparation, MAA; reviewing and editing, JAO, WZJ, H-BY and B-QF; Visualization, MAA, AH and RMAA; supervision, MZJ; project administration: W-ZJ and B-QF All authors have read and agreed with published version of the manuscript.

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