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Biochemical profile of cystic fluid of metacestodes in slaughtered food animals of Northern India

Sachin Patidar, Stuti Vatsya, Rajeev Ranjan Kumar and Jayshree Jakhar

Abstract
The present study was undertaken to analyse the biochemical profile of hydatid cyst fluid collected from slaughtered buffaloes and *Cysticercus* fluid collected from slaughtered sheep and goat in different districts in Uttarakhand (Udham Singh Nagar, Nainital, Bageshwar and Almora), Uttar Pradesh (Bareilly and Moradabad), Rajasthan (Alwar and Bharatpur) and Madhya Pradesh (Rewa and Ratlam). The biochemical profile of cystic fluid of 10 randomly collected hydatid cysts each from liver and lungs of buffaloes as well as 7 randomly collected *Cysticercus* each from sheep and goats were analyzed for various biochemical parameters. The overall mean ±SE concentration values of total protein (g/dl), cholesterol (mmol/L), glucose (mmol/L), creatinine (µmol/L), triglyceride (mmol/L), urea (mmol/L), ALT (U/L), AST (U/L), Ca (mmol/L), Na (mmol/L) and K(mmol/L) of hydatid cyst fluid (HCF) collected from liver of buffaloes were found to be 0.02±0.02, 0.14±0.06, 1.44±0.04, 49.59±1.27, 0.04±0.00, 5.08±0.22, 6088.33±1.78, 5712.31±1.90, 2.23±0.01, 111.46±1.84 and 7.68±0.06, respectively; collected from lungs of buffaloes were recorded to be 0.32±0.02, 0.13±0.03, 1.36±0.02, 49.12±1.28, 0.16±0.01, 6.84±0.40, 5241.32±2.80, 4286.06±2.17, 1.25±0.01, 81.37±3.04 and 7.23±0.37, respectively while the same for *Cysticercus* fluid collected from sheep were observed to be 0.70±0.03, 0.23±0.02, 3.76±0.13, 29.19±1.39, 0.21±0.02, 10.36±0.81, 9.47±1.65, 7.17±0.48, 4.38±0.47, 55.56±2.9 and 1.65±0.2, respectively and for *Cysticercus* fluid collected from goats were 0.72±0.02, 0.30±0.01, 3.17±0.13, 61.74±5.12, 0.19±0.01, 7.75±0.20, 7.12±0.30, 5.72±0.30, 5.41±0.25, 57.44±2.60 and 1.36±0.16, respectively. The biochemical analysis of metacestode fluid plays an important role in understanding the parasite's metabolism, physiology, and immunology, as well as its interaction with the host and can indicate variability in isolates from various places.

Keywords: Hydatid cyst, *Cysticercus*, fluid, biochemical parameters, buffaloes, sheep, goats

Introduction
Cestodes of the Taeniidae family infect the dog (the definitive host) and spread to a variety of intermediate host species, causing echinococcosis and cysticercosis. The larval tapeworms (metacestodes) grow as fluid-filled cysts in certain locations throughout the body. They act as space-occupying lesions and result in meat condemnation (Radostits *et al.*, 2007) [14]. *Echinococcus granulosus* is a tapeworm that causes echinococcosis/hydatidosis, a parasitic infection that affects both livestock and humans. Several strains of *Echinococcus* have been found, each with a different ability to infect intermediate hosts such as sheep, dogs, camels, pigs, humans, and horses. *Cysticercus tenuicollis* is a metacestode of the canine tapeworm *Taenia hydatigena* that lives on the visceral organs of sheep and goats such as the liver, spleen, lung, omentum, kidney, heart, etc. Adult *Taenia hydatigena* tapeworms are located in the intestines of final hosts such as dogs who can harbour tapeworms for up to a year (Smith and Sherman, 2011; Taylor *et al.*, 2007) [18, 19]. *Cysticercus tenuicollis* has a single invaginated scolex (bladderworm) with a long neck and is often found at meat inspection without any previous clinical signs (Kaufmann, 1996) [9]. The meat industry suffers significant losses as a result of the condemnation of edible organs such as liver (Wondimu *et al.*, 2011) [22]. It is also a direct nutritional loss for humans because the liver is high in vitamin A and glycogen.
Metabolic and biochemical variations may be the reasons for different strain development in *E. granulosus* (Thompson 1991; Shaafield *et al.* 1999) [20, 17]. In endemic locations where distinct transmission cycles occur, strain identification and differentiation should be performed using morphology, biology, biochemistry, immunology, and molecular methods (Kanwar *et al.* 1994; Kassis and Tanner 1977; Burgu *et al.* 2000) [8, 7, 3]. Biochemical measurements create a baseline evaluation of the chemical components of hydatid cyst fluid (HCF) that may be used to distinguish strain variants of *E. granulosus* in various places (Macpherson and McManusm, 1982; Shaafield *et al.*, 1999) [10, 17].

Very few researches on comparative biochemical analysis have been conducted in India to analyse the similarities and/or differences between the cystic fluids of sheep and goats, which might be relevant in determining *T. hydatigena* subspecies**.**

Since there is very little information available on the basic biochemical component of metacestodes infecting various hosts, so the biochemical profile of hydatid cysts obtained from the liver and lungs of buffaloes as well as *T. hydatigena* cysticerci collected from sheep and goats was compared.

Materials and Methods

Screening of animals for cyst

In the current study, a total of 456, 237 and 626 carcasses of buffaloes, sheep and goats, respectively were examined after routine slaughter in different animal slaughter house of Northern India {Uttarakhand (Udham Singh Nagar, Nainital, Bageshwar and Almora), Uttar Pradesh (Bareilly and Moradabad), Rajasthan (Alwar and Bharatpur) and Madhya Pradesh (Rewa and Ratlam)}from March 2021 to February 2022. The hydatid cysts were collected from liver and lungs of buffaloes whereas *Cysticercus* were collected from omentum, intestinal mesentery and liver of infected carcasses of sheep and goats slaughtered at different slaughter houses. The cysts were confirmed to be hydatid cyst and *C. tenuicollis* based on morphology, predilection site, size etc. Cysts were put in sterile cold container and transferred to the laboratory, Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar for further processing and examination. The biochemical profile of cystic fluid of 10 randomly collected hydatid cysts each from liver and lungs of buffaloes as well as 7 randomly collected *Cysticercus* each from sheep and goats were analyzed for various biochemical parameters.

Processing of cysts

For biochemical estimation, the surface of each cyst was cleaned with 70% alcohol. Then cystic fluid was aspirated aseptically with sterile syringe and transferred to clean test tubes and centrifuged for 30 minutes at 25200 x g at 4ºC. The supernatants thus obtained were evaluated for various biochemical parameters.

Biochemical analysis of hydatid cyst fluid (HCF) and *C. tenuicollis* fluid

The supernatants were evaluated for various biochemical parameters including glucose, total proteins, urea, triglycerides, cholesterol, creatinine, calcium, AST and ALT using (Erba®) Estimation Kit (Transasia Bio-medicals Ltd., Solan, H.P., India) and some parameters like sodium and potassium were estimated using (Beacon®) Estimation Kit (Beacon Diagnostics Pvt. Ltd., Navsari, India) following manufacturer's instructions.

Result and Discussion

Biochemical profile of hydatid fluid

The overall mean ±SE concentration values of total protein (g/dl), cholesterol (mmol/L), glucose (mmol/L), creatinine (µmol/L), triglyceride (mmol/L), urea (mmol/L), ALT (U/L), AST (U/L), Ca (mmol/L), Na(mmol/L) and K(mmol/L) of hydatid fluid collected from liver of buffaloes were 0.02±0.02, 0.14±0.06, 1.44±0.04, 49.59±1.27, 0.04±0.00, 5.08±0.22, 6088.33±1.78, 5712.31±1.90, 2.23±0.01, 111.46±1.84 and 7.68±0.06, respectively (Table 1).

The overall mean ±SE concentration values of total protein (g/dl), cholesterol (mmol/L), glucose (mmol/L), creatinine (µmol/L), triglyceride (mmol/L), urea (mmol/L), ALT (U/L), AST (U/L), Ca (mmol/L), Na(mmol/L) and K(mmol/L) of hydatid fluid collected from lungs of buffaloes were 0.32±0.02, 0.13±0.03, 1.36±0.02, 49.12±1.28, 0.16±0.01, 6.84±0.40, 5241.32±2.80, 4286.06±2.17, 1.25±0.01, 81.37±3.04 and 7.23±0.37, respectively (Table 1).

Total protein concentration of HCF collected from buffaloes was non-significantly higher in hydatid cyst fluid of lungs as compared to liver. In the present study, total protein was estimated by Biuret method which works on the principle that the peptide bonds of protein react with Cu^{++} ions in alkaline solution to form blue-violet complex (biuret reaction). Each Cu^{++} ions is complexed with 5-6 peptide bonds. Tartarate is added as a stabilizer and iodine is used to prevent auto reduction of alkaline copper complex (Tietz, 1986) [21]. The present findings are in agreement with those of Radfar *et al.* (2012) [15] and Yakchali *et al.* (2017).

Glucose concentration of hydatid cyst fluid was significantly higher in cyst fluid of liver as compared to lungs. Glucose was determined by Glucose Oxidase Peroxidase (GOD-POD) method (Tietz, 1986) [21] which is based on the principle that the glucose present in sample was oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The enzyme peroxidase catalyses the oxidative coupling of 4-amino antipyrine with phenol to yield a coloured quinoniemine complex whose absorbance was proportional to the concentration of glucose in sample. The present findings are in agreement with those of Radfar *et al.* (2012) [15].

Triglyceride concentration of hydatid cyst fluid was significantly higher in hydatid cyst fluid of cysts recovered from lungs of buffaloes as compared to liver. The present findings are in accordance with those of Radfar *et al.* (2012) [15] who also reported triglyceride concentration of cystic fluid was non-significantly higher in fluid of lungs as compared to liver.

Urea concentration was non-significantly higher in HCF of cysts obtained from lungs as compared to HCF from liver. The present findings are in agreement with those of Radfar *et al.* (2012) [15] who reported urea concentration of cystic fluid was non-significantly higher in fluid of lungs as compared to liver.

Cholesterol concentration of hydatid cyst fluid recovered from buffaloes was significantly higher in HCF collected from liver as compared to lungs. Cholesterol was estimated by Cholesterol Oxidase Phenol 4-Aminoantipyrine Peroxidase (CHOD-PAP) method which works on the principle that cholesterol esters in the presence of cholesterol esterase form cholesterol and fatty acids. Cholesterol oxidase oxidizes cholesterol to cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide reacts with phenol and 4- aminoantipyrine to form quinoniemine. The absorbance of quinoniemine was directly proportional to cholesterol concentration in sample (Roeschlau, 1974) [16].

Creatinine concentration of HCF was non-significantly higher in hydatid fluid of cysts recovered from liver as compared to lungs. The present findings are in contrast to those of Radfar *et al.* (2012) [15]. ALT and AST concentration of hydatid cyst fluid obtained from buffaloes was non-significantly higher in hydatid fluid of cysts collected from liver as compared to lungs. The present findings are in accordance with those of

Yakchali *et al*. (2017).

Calcium was determined by Arsenazo III method (Ferell, 1984) [5]. Arsenazo III combines with calcium ions at pH to form a coloured chromophore, absorbance of which is proportional to calcium concentration. The present findings are in agreement with those of Radfar *et al.* (2012) [15] and Yakchali *et al.* (2017). Potassium concentration of hydatid cyst fluid recovered from buffaloes was significantly higher in hydatid fluid of liver as compared to lungs.

Table 1: Biochemical profile of hydatid cysts collected from buffalo (n=10)

Biochemical parameters	Liver (mean±SE)	Lungs (mean±SE)	P value
Total Protein (g/dl)	0.02±0.02	0.32±0.02	0.898
Cholesterol (mmol/L)**	0.14±0.06^{a}	0.13±0.03^{b}	0.017**
Glucose (mmol/L)**	1.44±0.04^{a}	1.36±0.02^{b}	0.022**
Creatinine (µmol/L)	49.59±1.27	49.12±1.28	0.815
Triglyceride (mmol/L)*	0.04±0.00^{a}	0.16±0.01^{b}	0.004*
Urea (mmol/L)	5.08±0.22	6.84±0.40	0.065
ALT (U/L)	6088.33±1.78	5241.32±2.80	0.323
AST (U/L)	5712.31±1.90	4286.06±2.17	0.556
Ca (mmol/L)	2.23±0.01	1.25±0.01	0.519
Na (mmol/L)	111.46±1.84	81.37±3.04	0.074
K(mmol/L)*	7.68±0.06^{a}	7.23±0.37^{b}	0.001*
** a and b value bearing different alphabet and superscript in a row differ significantly (P<0.05) * a and b values bearing different alphabet and superscript in a row differ significantly (P<0.01)			

Biochemical profile of *Cysticercus* fluid

The overall values (mean ±SE) of total protein (g/dl), cholesterol (mmol/L), glucose (mmol/L), creatinine (µmol/L), triglyceride (mmol/L), urea (mmol/L), ALT (U/L), AST (U/L), Ca (mmol/L), Na (mmol/L) and K(mmol/L) of *Cysticercus* fluid collected from sheep were observed to be 0.70±0.03, 0.23±0.02, 3.76±0.13, 29.19±1.39, 0.21±0.02, 10.36±0.81, 9.47±1.65, 7.17±0.48, 4.38±0.47, 55.56±2.9 and 1.65±0.2, respectively (Table 2) while the same for *Cysticercus* fluid of cysts collected from goats were recorded to be 0.72±0.02, 0.30±0.01, 3.17±0.13, 61.74±5.12, 0.19±0.01, 7.75±0.20, 7.12±0.30, 5.72±0.30, 5.41±0.25, 57.44±2.60 and 1.36±0.16, respectively (Table 2).

Total protein concentration (g/dl) of *Cysticercus* fluid was non-significantly higher in *Cysticercus* collected from goats as compared to sheep. As compared to present study, Nazifi *et al.* (2011) [12] and Al-Bayati *et al.* (2012) [2] reported higher values of total protein concentration in *Cysticercus* fluid of sheep whereas Arunkumar and Krupakaran (2014) [1] reported higher values in *Cysticercus* fluid collected from goat. ALT and AST concentration (U/L) of *Cysticercus* fluid was significantly higher in cyst recovered from sheep as compared to goat. The results of present findings of ALT concentration are in accordance with those of Nath *et al.* (2010) [11] and Nazifi *et al.* (2011) [12] whereas in AST concentration values in the current study are in agreement with those of Nath *et al.* (2010) [11] and not with those of Nazifi *et al.* (2011) [12]. Cholesterol concentration (mmol/L) of *Cysticercus* fluid was significantly higher in cysts obtained from goat as compared to sheep whereas potassium and urea concentration (mmol/L) of cystic fluid was significantly higher in cyst recovered from sheep as compared to goat. Glucose, triglyceride and sodium concentration of *Cysticercus* fluid was non-significantly higher in cyst obtained from sheep as compared to goat whereas creatinine and calcium concentration of *Cysticercus* fluid was non-significantly higher in cysts collected from goat as compared to sheep. These observations are in agreement with those of Ouchene-Khelifi and Ouchene (2017) [13]. The variations in the values may be due to species, age and reproductive cycle of parasite.

Table 2: Biochemical profile of *Cysticercus* fluid collected from sheep and goat (n=7)

Biochemical parameters	Sheep (mean±SE)	Goat (mean±SE)	P value
Total Protein(g/dl)	0.70±0.03	0.72±0.02	0.489
Cholesterol(mmol/L)*	0.23±0.02^{a}	0.30±0.01^{b}	0.007*
Glucose(mmol/L)	3.76±0.13	3.17±0.13	0.880
Creatinine(µmol/L)	29.19±1.39	61.74±5.12	0.082
Triglyceride(mmol/L)	0.21±0.02	0.19±0.01	0.083
Urea(mmol/L)*	10.36±0.81^{a}	7.75±0.20^{b}	0.003*
ALT(U/L)*	9.47±1.65^{a}	7.12±0.30^{b}	0.000*
AST(U/L)	7.17±0.48	5.72±0.30	0.431
Ca(mmol/L)	4.38±0.47	5.41±0.25	0.161
Na(mmol/L)	55.56±2.90	57.44±2.60	0.852
K(mmol/L)*	1.65±0.20^{a}	1.36±0.16^{b}	0.000*
* a and b values bearing different alphabet and superscript in a row differ significantly (P<0.01)			

Metacestode fluid analysis is critical because it helps to understand the parasite's metabolism, physiology, and immunology, as well as its interaction with the host. Furthermore, it might suggest variability in isolates from different locations (Ganaie *et al.*, 2018) [6]. Analyzing biochemical components and factors in metacestode fluid that are involved in cyst fertility or infertility might help with parasite control by interrupting the transmission cycle and minimizing surgical risks. Because fluid exchanges materials with the host to allow protoscolices to survive and reproduce, analyzing the larval environment will aid in identifying the important components for parasite development and protoscolices' generation (fertility) (Fallah *et al.*, 2021) [4].

Conclusion

The biochemical analysis of metacestode fluid plays an important role in understanding the parasite's metabolism, physiology, and immunology, as well as its interaction with the host and can indicate variability in isolates from various places. Analysing biochemical substances in the metacestode fluid involved in cyst fertility or infertility can contribute in parasite management by halting the transmission cycle. The information generated in the present study can serve as baseline values to understand *Echinococcus*/*T. hydatigena* subspecies in the areas under study**.**

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