

A STUDY ON BIOCHEMICAL PROFILES OF HYDATID CYST FLUIDS FROM RUMINANTS AND HUMANS IN PUNJAB, PAKISTAN

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ABSTARCT

Echinococcus granulosus, a cestode parasite dwells in small intestine of canids and has intermediate hosts such as livestock and humans. Its infection in intermediate hosts results in hydatidosis. The objective of the study was to determine and analyse the biochemical profile manifested by hydatid cyst fluid (HCF) of various ruminants and humans. The fluid was aspirated from fertile and sterile cysts of infected sheep, goat, camel, buffalo, humans and subsequently subjected to biochemical analysis by Randox and Roche kits. The results revealed that in fertile isolates maximum values (mg/dl) of glucose (100.91±6.63), cholesterol (201.21±1.26), triglyceride (192.4±1.83), potassium (119.85±0.49) were recorded in sheep; calcium (10.18±0.24), chloride (107.51±0.37), urea (67.58±0.44) in cattle; protein (g/l) (111.3±5.32), copper (41.24±1.38), urea (73.08±3.68) in camel, Uric acid (13.01±0.36), creatinine (0.20±0.01), magnesium (9.2±0.24) and sodium (122.3±0.64) in human. In sterile isolates highest values of protein (118.39±8.78), magnesium (7.75±0.35) were observed in sheep; calcium (10.2±0.26), potassium (7.83±0.36), sodium (197.23±0.73) in cattle; calcium (7.91±0.23), triglyceride (155.01±8.76), creatinine (0.03±0.01), sodium (19.05±1.22) in buffalo; uric acid (10.69±0.25), glucose (118.97±0.31), urea (372.93±8.92), cholesterol (201.73±2.11), triglyceride (183.39±1.93) in camel and creatinine (0.10±0.01) in human. Hence, biochemical profiles from fertile and sterile isolates showed significant differences among ruminants and human isolates and these parameters can be employed as diagnostic approach for identification of infection.

Key words: *Echinococcus granulosus*, biochemical, humans, ruminants, fertile and sterile cysts.

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INTRODUCTION

Hydatidosis is a neglected zoonotic disease and global public health problem caused by *Echinococcus granulosus* (Taherkhani *et al.*, 2000; Shafiq *et al.*, 2005; Torgerson *et al.*, 2006; Esatgil *et al.*, 2007; Tavakoli *et al.*, 2008; Shafiq *et al.*, 2009). The annual infection rate is 1.2 million in the world (Craig *et al.*, 2007). It is mainly endemic to Asia particularly in Pakistan & India, South America, Middle East and Australia (Pednekar *et al.*, 2009). The infection rates among dairy animals are aggravating day by day in certain provinces (KPK) of Pakistan: buffaloes (15.88%) followed by cows (15.79%), sheep (15.38%), and goats (3.25%) (Haleem *et al.*, 2018).

Echinococcus granulosus is a tapeworm which causes a parasitic disease, echinococcosis/ hydatidosis that affects various livestock and humans. Various strains of echinococcus have been identified with differential potential to infect the intermediate hosts like sheep, dog, camel, pig, human, and horse. The modern phylogenetic systematics has recognized nine different species of *Echinococcus* (Nakao *et al.*, 2013). The parasites are

found attached to the mucosa of the small intestine in the canids (definitive hosts like dogs, wolves and foxes) where they develop into adult stage. In definitive hosts, the eggs are produced within 4–6 weeks of infection which latter on are passed out with faeces. The eggs after ingestion by the intermediate host reach the liver, lungs and other viscera through blood or lymph where cyst development begins (Farmer *et al.*, 1990; Zhang *et al.*, 2003; Siracusano *et al.*, 2012 a, b). The eggs develop in to oncosphere larvae which remain alive for several years regardless of the host immune system. The larvae are characterized by long-term growth in the intermediate host (Zhang *et al.*, 2003). The oncospheres penetrate the intestinal mucosa and reach the liver via the blood stream. A cyst develops in the infected organ after variable time period (Eckert *et al.*, 2001). The hydatid cyst is unilocular, filled with hydatid fluid which might be fertile or sterile. Fertile cysts containing protoscoleces parasites are maintained in the host by developing a protective mechanism against the immunological and physiological reactions occurring in the host (Diaz *et al.*, 2000). Hydatid fluid has a complex mixture of excretory

and secretory products resulting from protoscolecocytes and the germinal layer which armed the parasite with antigenic properties (Siracusano *et al.*, 2009). Parasite molecules lead to the chronic establishment of the infection and interfere with the functional activity of the host's immune cells (Hewitson *et al.*, 2009).

Analysis of hydatid cysts have performed for electrolytes, enzymes, nucleic acids, proteins, nitrogenous waste products, carbohydrates and lipids (Zeghir-Bouteldja *et al.*, 2017). Currently, chromatographic method for enrichment of Antigen 5 and exosomal signatures derived from extracellular vesicles confined in cyst and are being employed as analytical tools for hydatid cyst fluid (Siles-Lucas, 2017). Determination of the chemical and biochemical parameters in hydatid cyst fluids of infected sheep and cattle may help to identify the source of human infection. Statistically significant differences in the fluid of hydatid cyst collected from sheep, goat, camel, cattle and human for comparative study on the biochemical parameters in Iran has been reported (Radfar *et al.*, 2004). Quantitative deviations in the levels of glucose, calcium and creatinine in the cystic fluids of camels, sheep, goat, cattle and humans were detected.

Keeping in view the escalating prevalence of hydatidosis, unawareness on the part of farmers and lack of appropriate approaches for its diagnosis, it is crucial to have preliminary information on biochemical analysis of both fertile and sterile hydatid cyst fluids from different species of animals and human beings by using various biochemical kits. Data obtained from comparative study of various biochemical parameters may indicate some common values for precise finding of hydatidosis in ruminants and humans.

MATERIALS AND METHODS

The present study was approved by ethical committee of primary author's university. Cysts were obtained from various animals such as cow, sheep, camel, and goat slaughtered in different abattoirs of Punjab. A total of 39,738 slaughtered animals were examined for hydatid cysts during postmortem inspection including 5300 buffaloes, 15,857 sheep, 15,001 goats, 2990 cattle and 590 camels. The hydatid cysts were obtained from liver and lungs of host animals. Each cyst was washed with distilled water and hydatid cyst fluid (HCF) was aspirated with sterile syringe with hypodermic needle No.18G. Hydatid cyst fluids were also collected from humans who were operated for hydatidosis in different hospitals of Punjab. All fluid samples were centrifuged at 15000 rpm at 4°C for 30 minutes and supernatants were analysed for various biochemical parameters (Radfar *et al.*, 2004). Ten samples were selected from each animal species isolate and 10 from human isolates (fertile and sterile HCF). The biochemical parameters which are

considered indicative of most of the diseases were analysed in the present study.

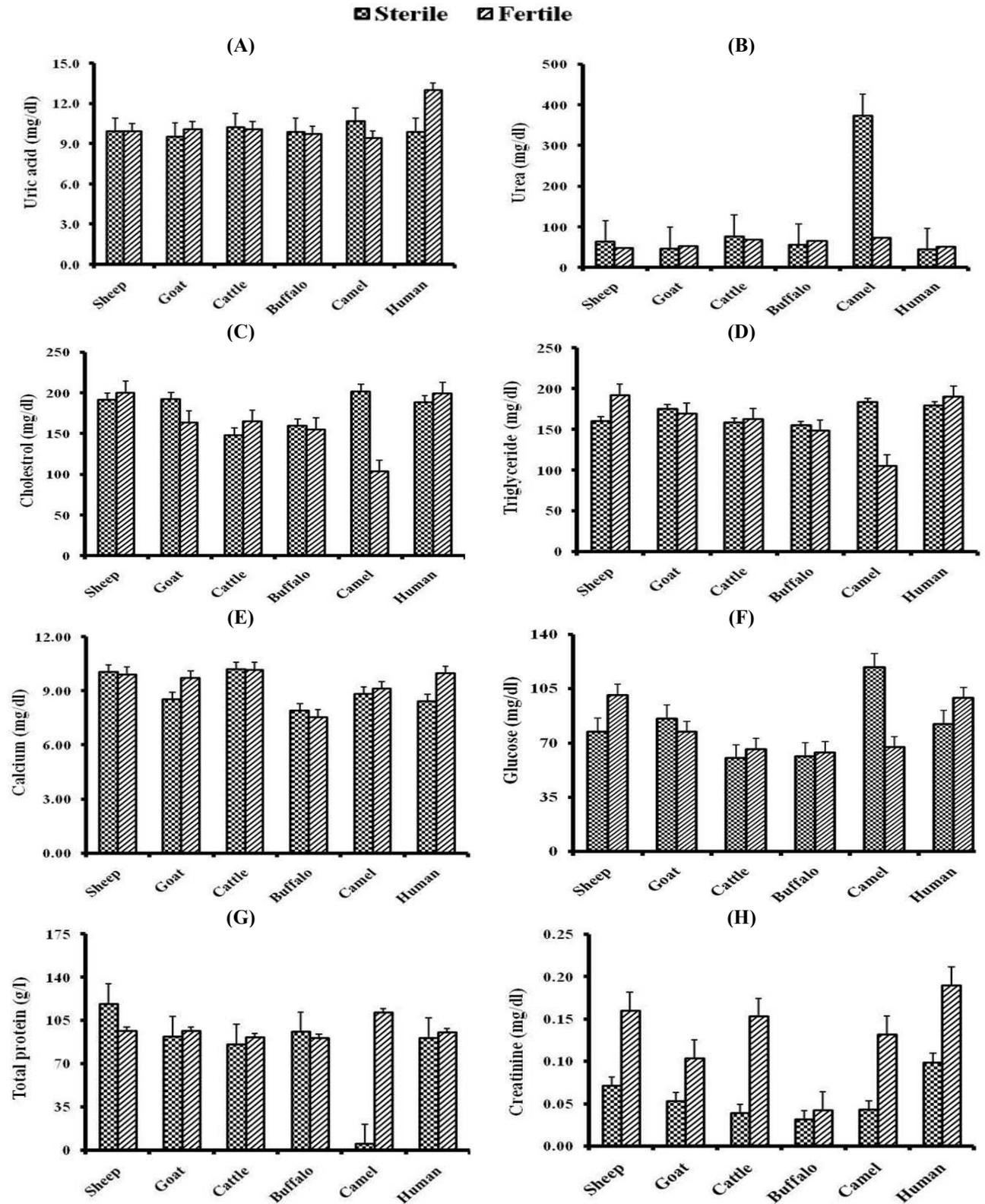
Estimation of urea (mg/dl), cholesterol (mg/dl), triglyceride (mg/dl), calcium (mg/dl), uric acid (mg/dl), glucose (mg/dl), total protein (g/l) and copper (mmol/l) were determined by using the RANDOX Kit according to the manufacturer's instructions. Estimation of magnesium (mg/dl) and creatinine (mg/dl) (Jaffe' method) were determined by using the Roche Kit according to the manufacturer's instructions. Roche automate 912 analyser was used to calculate the concentration of each sample. Fluid samples were evaluated for potassium (mmol/l), sodium (mmol/l) and chloride (mmol/l) electrolytes in Easy Lyte plus Na/K/Cl. (MEDICA). Sample size was kept at 100µl and analysed for 60 seconds at 15-32°C and 85% humidity. Statistical analysis was done by Multivariate analysis (MNOVA) using SPSS 13.0.

RESULTS

Calcium (mg/dl) level was higher in fertile (10.18±0.24) and sterile (10.2±0.26) isolates of cattle and lower in fertile (7.56±0.10) and sterile (7.91±0.23) isolates of buffalo. Cholesterol (mg/dl) level was higher in fertile isolates of sheep (201.21±1.26) and lower in camel (103.23±0.75), while higher in sterile isolates of camel (201.73±2.11), and lower in cattle (148.05±6.55). Experimental study revealed, total protein (g/l) was higher in fertile isolates of camel (111.3±5.32) and less in buffalo (90.92±0.65), while maximum in sterile isolates of sheep (118.39±8.78) and lower in camel (5.04±0.65). Observed values showed that triglycerides (mg/dl) was higher in fertile isolates of sheep (192.41±1.83) and less in camel (105.50±3.29), while maximum in sterile isolates of camel (183.39±1.93) and lower in buffalo (155.01±8.76). Level of magnesium (mg/dl) was higher in fertile isolates of human (9.20±0.24) and less in camel (0.30±0.02), while maximum in sterile isolates of sheep (7.76±0.35) and lower in buffalo (0.08±0.01). Estimated value of potassium (mmol/l) was higher in fertile isolates of sheep (119.85±) and less in buffalo (5.21±0.51), while maximum in sterile isolates of cattle (7.83±0.36) and lower in buffalo (1.40±0.05). Uric acid (mg/dl) level in fertile groups was more in human (13.01±0.036) and less in camel (9.45±0.15), while in sterile groups the value was higher in camel (10.69±0.25) and lower in goat (9.56±0.13). Copper (mmol/l) level was higher in fertile isolates of camel (41.24±1.38) and less in buffalo (21.17±1.51), while maximum in sterile isolates of goat (37.46±1.42) and lower in camel (15.47±0.92). Glucose (mg/dl) level was maximum in fertile isolates of sheep (100.91±6.63) and less in buffalo (63.92±0.80), while in sterile isolates was higher in camel (118.97±0.31) and lower in cattle (60.19±0.45). Creatinine (mg/dl) was higher in fertile isolates of human (0.20±0.01) and less in

buffalo (0.04 ± 0.01), while maximum in sterile isolates of human (0.10 ± 0.01) and lower in buffalo (0.03 ± 0.01). Urea (mg/dl) level was higher in fertile groups of camel

(73.08 ± 3.68) and less in sheep (47.34 ± 0.79), while in sterile groups values were higher in camel (372.9 ± 8.92) and lower in human (43.72 ± 0.29).



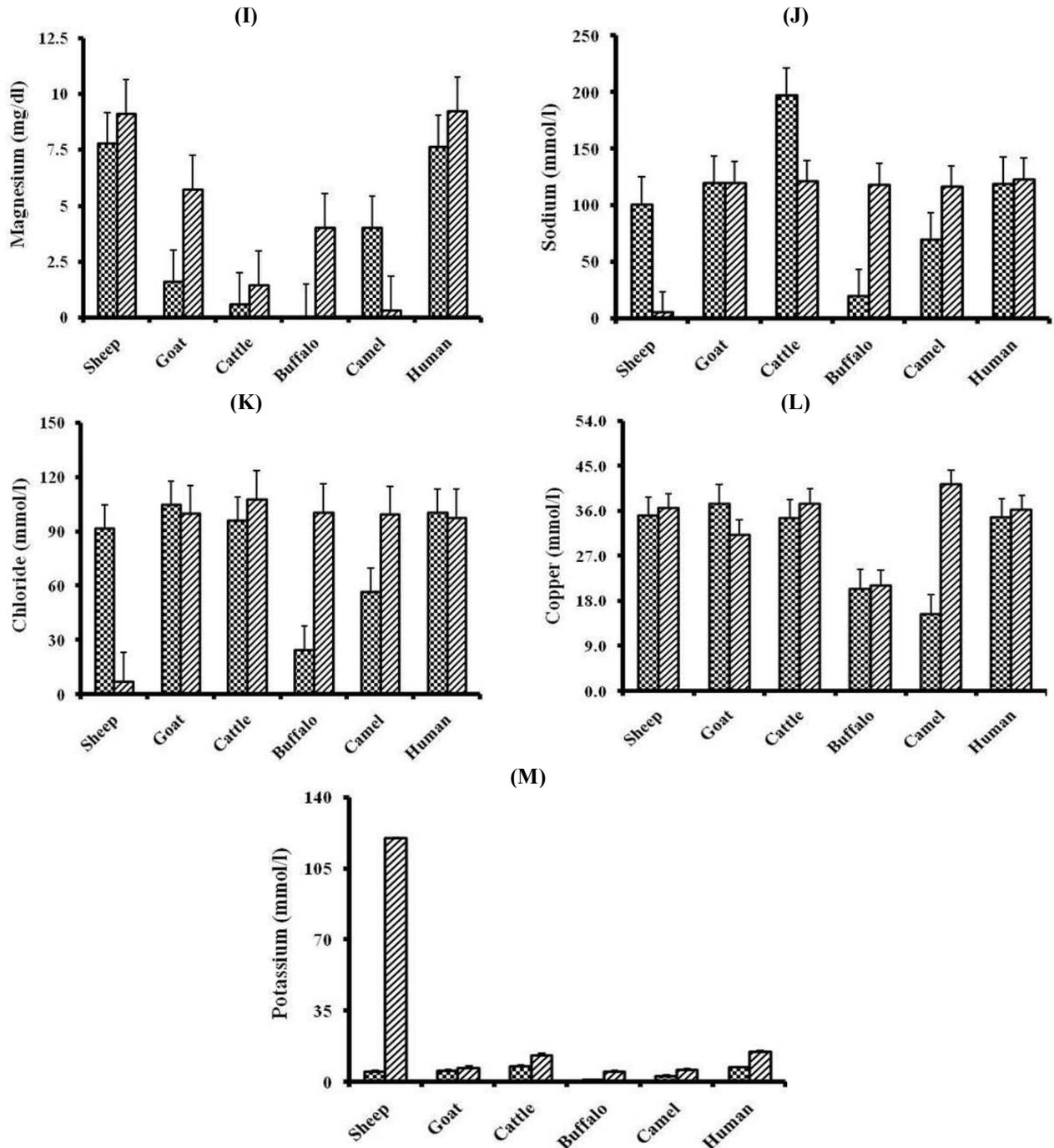


Fig. 1: Bar graph shows variation in biochemical profiles (mean±SE) of fertile and sterile hydatid cyst fluids from various animals and human isolates

(A) Uric Acid (mg/dl); (B) Urea (mg/dl); (C) Cholesterol (mg/dl); (D) Triglyceride (mg/dl); (E) Calcium (mg/dl); (F) Glucose (mg/dl); (G) Total Protein (g/l); (H) Creatinine (mg/dl); (I) Magnesium (mg/dl); (J) Sodium (mmol/l); (K) Chloride (mmol/l); (L) Copper (mmol/l); (M) Potassium (mmol/l).

Chloride (mmol/l) contents were higher in fertile isolates of cattle (107.51 ± 0.37) and less in sheep (7.33 ± 0.43), while maximum in sterile isolates of goat (104.48 ± 0.51) and lower in buffalo (24.51 ± 0.86). Sodium (mmol/l) level were found higher in fertile isolates of human

(122.30 ± 0.64) and less in sheep (4.52 ± 0.30), while maximum in sterile isolates of cattle (197.23 ± 0.73) and lower in buffalo (19.05 ± 1.22) (Fig. 1). All values were significantly different ($p < 0.05$).

DISCUSSION

Hydatidosis is a zoonotic disease affecting the dairy animals and humans on a large scale globally. A limited data regarding prevalence of hydatidosis is available from Sindh, Punjab and Khyber Pakhtunkhwa in Pakistan (Nadeem *et al.*, 2006). The reported incidence rate of hydatidosis in various region of Pakistan from 1989 to 2015 was between 7.19 and 24.40% in buffaloes (Latif *et al.*, 2010), 2.44 and 35% in cattle (Latif *et al.*, 2010; Ali *et al.*, 2015) and 2.44 and 6.61% in goats (Latif *et al.*, 2010; Iqbal *et al.*, 2012).

The biochemical profiles from fertile and sterile isolates showed significant differences among all animal species and human isolates. The principal strain which causes infection in human is sheep strain (Thompson *et al.*, 1995). Similar to our findings of potassium (mmol/l) values for sheep (sterile), camel (fertile) and goat (fertile) were reported by other authors (Thompson *et al.*, 1995; Izadi and Ajami, 2006). Our results of sodium (mmol/l) values for goat (sterile), human (fertile) and cattle (fertile) were comparable with the results reported by Izadi and Ajami (2006). Glucose, cholesterol, triglyceride and urea values found in present investigations were lower and creatinine were higher in cattle, sheep, goat, camel and human isolates as compared to the results of Izadi and Ajami (2006) and Juyi, (2013). Values of calcium for sheep (sterile), camel (fertile) and cattle (fertile, sterile) found in our results were nearly similar to the other reports. The higher values of sodium maintain that these elements enter the cyst to promote the growth of the parasites (Izadi and Ajami, 2006; Juyi, 2013).

In sheep (fertile) isolates, our results for Ca, Mg, Na, K were different as compared to the Rahdar *et al.* (2008). Ca ions maintain the pH of the cyst and hence resist the development of acidity in it. Moreover, Ca and Mg ions are found in the calcareous bodies of the cyst (Frayha, *et al.*, 1980). Total protein (g/l), copper (mmol/l), triglyceride levels for fertile and sterile isolates showed significant difference among human and animal species $p < 0.05$, our values were different from those reported by Radfar and Iranyar (2004) and Izadi and Ajami (2006). Copper levels for fertile and sterile groups of sheep were lower than the values reported by Kojouri and Moshtaghi (2008). The levels reported by Rashid *et al.* (2013) for glucose, triglycerides, calcium varied from our work but sodium, potassium, chloride were nearly similar in all sterile samples of sheep and cattle. Buffalo species is endemic to India and Pakistan, so detailed report on this study could not be found from the literature available. Present analysis showed fertile and sterile isolates of buffalo revealed significant differences in urea, triglyceride, calcium, cholesterol, uric acid, potassium, magnesium, copper and insignificant difference in the values of chloride, creatinine, sodium, glucose and total protein.

Conclusion: Present study revealed significant variation of biochemical parameters in different hydatid cyst fluids of various intermediate hosts (animals and human) isolates. Variation in composition of hydatid cyst fluids in different species of animals and human may be due to multiple strains of *E. granulosus* in Punjab, Pakistan. This analysis will be further useful for immunological studies, diagnostic tests and may be helpful to find out various strains of *E. granulosus* in Punjab by using PCR technique.

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