Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 1021-1025

Received: 26-03-2019
Accepted: 28-04-2019

Omer Mohi U Din Sofi
Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Stuti Vatsya
Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Rajeev Ranjan Kumar
Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

AK Upadhyay
Department of Veterinary Public Health and Epidemiology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Seema Agrawal
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Correspondence
Stuti Vatsya
Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Micrometry of hooks of protoscolices of *Echinococcus granulosus* G5 genotype recovered from buffaloes in northern part of India

Omer Mohi U Din Sofi, Stuti Vatsya, Rajeev Ranjan Kumar, AK Upadhyay and Seema Agrawal

Abstract
Considering the zoonotic importance of *Echinococcus granulosus*, micrometry of the hooks of protoscolices of *E. granulosus* (G5 genotype) was done to develop a baseline data and use it as an alternative tool to identify this genotype. Micrometric study (Mean±SE) of large and small hooks of protoscolices of *E. granulosus* (G5 genotype) recovered from buffaloes in northern part of India was performed by taking seven 7 parameters *viz.* total hook length (TL), blade length (BL), blade width (BW), blade guard distance (BGD), handle length (HL), handle width (HW) and total width (TW) {n=25; large hooks (TL=21.66±0.71µm, BL=13.96± 0.62µm, BW=3.94± 0.47µm, BGD=10.99± 0.51µm, HL=7.05± 0.51µm, HW=3.28± 0.46µm, TW=7.15± 0.54µm), small hooks (TL=17.23± 0.55µm, BL=9.23± 0.46µm, BW=2.25± 0.41µm, BGD=7.16± 0.45µm, HL=7.44± 0.33µm, HW=3.29± 0.52µm, TW=6.12± 0.47µm)}. Overall length and handle length of massive and small hooks of protoscolices of *E. granulosus* (G5 genotype) of buffalo isolates were observed to be higher. The baseline data generated in the study can be used it as a tool to identify this genotype.

Keywords: *Echinococcus granulosus*, protoscolices, hooks, micrometry

Introduction
Echinococcosis has a serious negative effect on potential of productivity of animals since the period of Hippocrates (Gemmell and Roberts, 1998) [12]. While hydatidosis occurs due to contamination with the larval stage i.e. metacestode, echinococcosis implies disease with both adult and larval contaminations (NICD, 2005) [20]. Cystic echinococcosis (CE) and alveolar echinococcosis (AE) are two most essential types of echinococcosis which are of restorative and general wellbeing significance in people (WHO, 2017) [30]. The various species of genus *Echinococcus* with genotypes causing cystic echinococcosis (CE) includes the *E. granulosus sensu stricto* (s.s.) (G1/G2/G3), *E. granulosus sensu lato* (s.l.) complex groups, *E. canadensis* (G6/G7/G8/G10), *E. ortleppi* (G5), *E. equines* (G4) and *E. felidis* (lion strain) (Cucher *et al*., 2016) [10]. At least 10 strains (G1–10) of *E. granulosus* s.l. have been recognized forming 4 major clades (G1–G3, G4, G5 and G6 to G10) (Nakao *et al*., 2007) [19] all of which have variable range of hosts, ability to infect host and genetic characteristics (Eckert *et al*., 2001) [11]. Taxonomic correction of G1 to G5 as *E. granulosus sensu stricto* (G1 to G3), *E. equinus* (G4) and *E. ortleppi* (G5) has been proposed through the ongoing re-assessments of *Echinococcus* species (Ito *et al*., 2007) [15]. Solid proof exists for species status of genotypes G6 to G10 (*E. canadensis*) and the lion strain (*E. felidis*). Natural varieties in *E. granulosus* impact its life cycle designs,
pathogenesis caused in host, immunological results in host, capacity to transmit the disease and the response to various drugs. For instance, *E. equinus*, *E. granulosus s.s*, *E. canadensis* and *E. ortleppi* are transmitted essentially through domestic life cycles (Carmena and Cardona, 2014) [7]. This reinstates the fact that *Echinococcus* species identification is important as it may affect the designated developing and evaluation of prevention and control measures, diagnostic assays and therapeutics (Thompson and McManus, 2002 and McManus, 2010) [27,18].
Prior examinations about the strains of *E. granulosus* in animals of Eastern India exhibited the prevalence of four genotypes G1, G2, G3 and G5 (Bhattacharya *et al*., 2008) [6]. G1, G2 and G3 genotypes have been secluded from domesticated animals of West Bengal (Craig *et al*., 2007) [9].

Four unique genotypes i.e. G1, G2, G3 and G5 genotype have been secluded from food producing animals in Maharashtra and bordering region of Western India (Pednekar *et al*., 2009) [21] while as from North India, G1 and G3 genotypes have been exhibited from domesticated animals (Singh *et al.*, 2012) [24]. G1 and G3 genotypes have zoonotic potential and are additionally prevalent genotypes affecting people in India. G3 genotype has been isolated from Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir and G5 genotypes have been isolated from the patients of Uttarakhand (Sharma *et al*., 2013) [23]. In India, not very much reports are accessible in regards to genotypes of *E. granulosus* tainting animals in various parts of the nation *viz.* Eastern India (Bhattacharya *et al*., 2008) [6], Mumbai, Maharashtra (Pednekar *et al*., 2009) [21] and Northern India (Singh *et al*., 2012) [24].

Despite genotyping, different workers from various nations have included micrometry of the hooks of protoscolices of *E. granulosus* as a tool to mark identitification between various strains of *Echinococcus viz.* Iran (Karimi and Dianatpour, 2008) [17], Argentenia (Andresiuk *et al*., 2013) [5] and India (Gholami *et al*., 2018) [13]. Measuring factors of the hooks include total number of hooks (NH), total length of hooks (TL), total width of hooks (TW), length of the blade (BL), width of the blade (BW), length of the handle (HL), width of the handle (HW) and distance between blade and guard (BGD). Considering the zoonotic importance of the parasite, micrometry of the hooks of protoscolices of *E. granulosus* (G5 genotype) was done to develop a baseline data and use it as an alternative tool to identify this genotype.

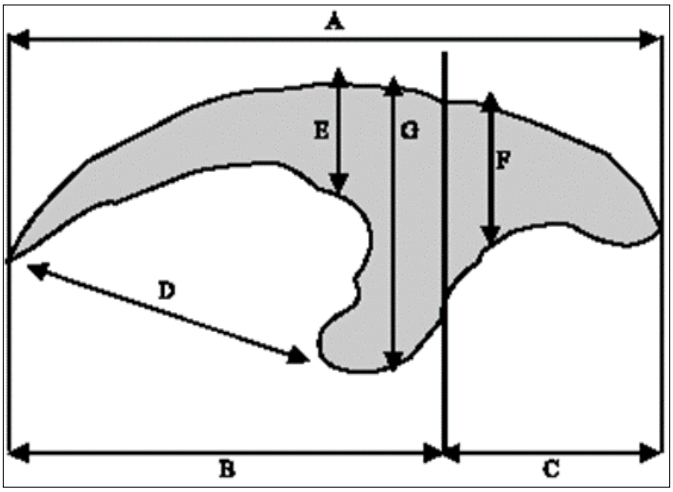
Materials and Methods

The present study was conducted in the Department of Veterinary Parasitology, College of Veterinary and Animal Sciences., Govind Ballabh Pant University of Agriculture and Technology, Pantnagar and in and around two districts of (U.S. Nagar and Nainital) northern part of India. In a previous study, the DNA and *cox1* gene amplification fragment length of all the 25 *Echinococcus granulosus* isolates (protoscolices and laminated layer) was found to be 18kb and 493bp, respectively. The *cox1* gene sequence obtained from Udham Singh Nagar and Nainital isolates showed 100% and 99.9% identity with G5 genotype, respectively which confirmed the prevalence of G5 genotype of *E. granulosus* in buffaloes in the study area.

Micrometry of hooks of protoscolices

The morphological studies were carried out on the rostellar hooks of protoscolices isolated from liver and lungs of buffaloes naturally infected with *E. granulosus*. Morphological characteristics of larval (protoscolices) hooks were analyzed in 20 samples from buffalo lungs, 12 samples from buffalo liver, 07 samples from mixed liver and lungs. In total, 25 protoscolices were selected from each sample and the total number of hooks were counted. In this study, only complete row of hooks were measured, whereas, incomplete row of hooks were not measured as discussed by Sweatman and Williams, (1963) [25].

Hooks of the protoscolices of *E. granulosus* were isolated from protoscolices by low amplitude sonication and the isolated hooks (small and large) were characterized by micrometry as described by Gholami *et al*. (2018) [13]. The micrometry was done at 100X with scale 1 division of ocular = 0.714 micrometer. A total of 7 parameters were taken for the measurement of both small and large hooks *viz.* total hook length (TL), blade length (BL), blade width (BW), blade guard distance (BGD), handle length (HL), handle width (HW) and total width (TW) (Figure 1).



A Total length (TL)
B Blade length (BL)
C Handle length (HL)
D Blade guard distance (BGD)
E Blade width (BW)
F Handle width (HW)
G Total width (TW)

Fig 1: Diagramatic representation of hook micrometric characters (adapted from Gholami *et al.*, 2018)

Results and Discussion

Morphology of protoscolices

The morphological studies were carried out on the rostellar hooks of protoscolices isolated from liver and lungs of buffaloes naturally infected with *E. granulosus* (Table 1 and 2; Figure 2). The average of different morphological parameters were as follows- total length of large hooks of protoscolices was measured as 21.66±0.71µm with a total width as 7.15± 0.54µm; blade length, 13.96± 0.62 µm; blade guard distance, 10.99± 0.51µm, blade width, 3.94± 0.47µm, handle length, 7.05± 0.51µm and handle width 3.28± 0.46µm. The total length of small hooks of protoscolices was measured as 17.23± 0.55µmwith a total width as 6.12± 0.47µm; blade length, 9.23± 0.46µm; blade guard distance, 7.16± 0.45µm; blade width, 2.25± 0.41µm, handle length, 7.44± 0.33µm and handle width 3.29± 0.52µm (Table 3).

Table 1: Rostellar hook (Large Hooks) characteristics of the protoscolices of *E. granulosus* cysts from buffaloes (Micrometry at 100X with scale 1 dice of ocular = 0.714 micometer) (n=25)

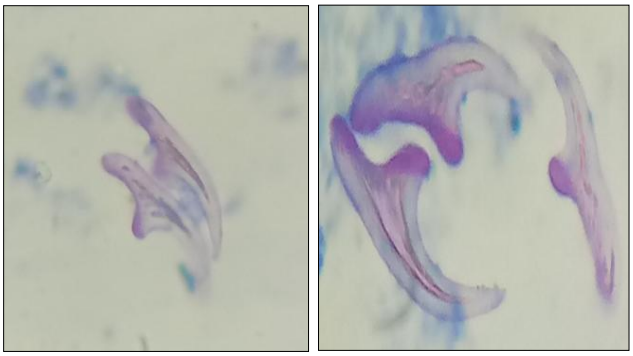
Sample no.	TL (µm)	TW (µm)	BL (µm)	BGD (µm)	BW (µm)	HL (µm)	HW (µm)
01	21.42	7.14	14.28	10.71	3.57	6.42	2.85
02	21.33	6.99	14.22	10.11	3.98	6.67	2.98
03	21.63	7.62	14.87	10.34	3.24	6.89	2.45
04	21.44	6.87	14.74	10.89	3.87	6.53	3.98
05	20.59	6.32	13.43	11.32	3.19	7.11	2.54
06	22.12	7.97	13.98	11.54	4.12	7.35	2.89
07	22.76	7.68	14.33	10.87	4.35	6.45	3.44
08	20.57	7.91	13.67	11.78	3.28	6.32	3.21
09	20.76	6.89	14.54	10.43	4.29	7.23	3.32
10	21.66	6.43	14.23	10.58	3.14	7.19	3.98
11	21.28	6.22	14.78	11.76	3.95	6.32	3.45
12	21.56	6.54	13.68	10.44	3.68	7.24	3.65
13	21.88	6.82	14.28	10.53	3.92	7.77	3.88
14	21.76	6.25	14.63	10.72	3.55	7.64	3.91
15	22.11	7.48	14.95	10.92	3.91	7.52	3.11
16	22.64	7.53	14.22	10.35	3.88	7.21	2.95
17	22.45	7.39	13.33	11.15	3.49	6.28	3.81
18	22.62	7.28	13.91	11.32	4.11	6.23	3.33
19	22.91	7.77	13.88	11.24	4.29	6.88	2.88
20	21.33	7.92	12.99	11.59	4.51	7.11	2.61
21	20.98	6.81	13.44	11.72	4.82	7.82	2.91
22	20.87	6.96	13.66	10.77	4.15	7.29	3.11
23	20.64	6.91	13.26	10.93	4.95	7.73	3.82
24	22.51	7.29	12.89	10.98	4.16	7.71	3.44
25	21.84	7.88	12.83	11.98	4.27	7.42	3.59

TL=Total Length, TW=Total Width, BL= Blade Length, BD= Blade guard distance, BW= Blade Width, HL= Handle Length, HW= Handle Width

Table 2: Rostellar hook (Small Hooks) characteristics of the protoscolices of *E. granulosus* cysts from buffaloes (Micrometry at 100X with scale 1 dice of ocular = 0.714 micometer) (n=25)

Sample no.	TL (µm)	TW (µm)	BL (µm)	BGD (µm)	BW (µm)	HL (µm)	HW (µm)
01	17.85	5.71	9.98	7.14	2.14	7.85	2.49
02	17.63	6.23	9.76	7.39	2.45	7.56	2.98
03	17.83	6.58	9.65	7.46	2.32	7.63	2.45
04	17.98	5.95	8.77	6.98	2.42	7.76	3.11
05	16.55	5.67	8.97	7.53	2.85	7.11	2.54
06	16.39	5.43	8.67	7.11	2.64	7.35	2.89
07	16.86	6.23	8.45	7.65	2.44	6.97	3.24
08	16.43	5.43	8.96	7.95	2.54	6.84	3.21
09	16.96	5.31	9.23	6.96	1.78	7.23	3.32
10	17.32	6.42	9.57	6.78	1.93	7.19	2.43
11	17.99	6.22	9.12	6.43	1.23	6.77	2.16
12	17.39	6.54	9.54	6.88	1.67	7.14	3.29
13	17.66	6.29	9.86	6.85	2.81	7.25	3.82
14	17.62	6.48	9.88	6.89	2.52	7.82	3.29
15	17.68	6.49	9.41	6.92	2.11	7.71	3.76
16	17.89	6.77	9.27	6.98	1.97	7.91	3.94
17	17.82	6.85	9.82	6.26	1.95	7.96	3.81
18	17.77	6.72	9.71	7.77	1.92	7.52	3.88
19	16.65	6.51	8.88	7.62	2.61	7.41	3.61
20	16.77	6.27	8.71	6.69	2.47	7.45	3.65
21	16.81	6.19	8.76	6.51	2.22	7.77	3.48
22	16.92	5.88	8.59	7.25	2.98	7.67	3.68
23	16.88	5.97	8.69	7.77	1.82	7.58	3.71
24	16.45	5.41	9.11	7.52	2.52	7.28	3.77
25	16.66	5.39	9.33	7.66	1.98	7.21	3.83

TL=Total Length, TW=Total Width, BL= Blade Length, BD= Blade guard distance, BW= Blade Width, HL= Handle Length, HW= Handle Width



(a) (b)

Fig 2: Photograph showing hooks of protoscolices stained with acid fast stain 40X (a) and 100X (b)

Table 3: Micrometric characters of large and small hooks (Mean ± S.D)

Hook type	TL (µm)	TW (µm)	BL (µm)	BGD (µm)	BW (µm)	HL (µm)	HW (µm)
Large	21.66± 0.71	7.15± 0.54	13.96± 0.62	10.99± 0.51	3.94± 0.47	7.05± 0.51	3.28± 0.46
Small	17.23± 0.55	6.12± 0.47	9.23± 0.46	7.16± 0.45	2.25± 0.41	7.44± 0.33	3.29± 0.52

TL=Total Length, TW=Total Width, BL= Blade Length, BD= Blade guard distance, BW= Blade Width, HL= Handle Length, HW= Handle Width

The large and small hooks in the rostella were present in two rows in alternate fashion and had entire outline in all of the samples. About 25% of the samples of buffaloes had been determined to have both small hooks in among the massive hooks or large hooks in between the small hooks. Overall length and handle length of each massive and small hook were observed to be substantially higher. Moreover, variations existed within the curvature of blades which were found to be sharp and much less curved. In case of paired massive hooks, the space among the 2 hooks was generally the same as the distance discovered generally among the massive and small hooks, whereas, when small hooks had been paired they had been normally closer collectively than regular. Further, variations in the overall length, blade length, blade width and curvature of blades of hooks were also recorded.

Rostellar hook morphology remains into consideration to be a legitimate criterion for differentiating *E. granulosus* strains (Thompson *et al*., 1984; Ponce Gordo and Cuesta Bandera, 1997; Gemmell *et al*., 1998; Ahmad *et al*., 2001; Tashani *et al*., 2002; Jenkins *et al*., 2005; Ahmadi and Dalimi, 2006; Almeida *et al*., 2007) [29, 22, 12, 1, 26, 16, 3, 4]. It is relatively a short and less expensive approach of strain characterization, especially for epidemiological investigations. In such manner, the total numbers, total length and blade length of rostellar snares were proposed as imperative tools for differentiation of strains (Ponce Gordo and Cuesta Bandera, 1997) [22]. Consequently, our findings additionally bolstered this previous information that overall length and handle length are the most critical characters and in this way may be used extra reliably for differentiating *E. granulosus* strains from various intermediate host species. By means of the fact that the larval hook is firmly placed within the adult hook and stays unchanged because it passes via the final host (Hobbs *et al*., 1990) [14], it is far likely that larval hook characteristics can be utilized to conclude the intermediate host origin of adult worms and could be beneficial for figuring out transmission styles of the distinct strains (Constantine *et al*., 1993 and Ahmadi, 2004) [8, 2].

In lots of these researches, 30 and 10 protoscolices have been used for each sample for figuring out the total number and measuring characters of hooks, respectively, and normally two large and two small hooks (2+2) were measured from each rostellum. Ponce Gordo and Cuesta Bandera (1997) [22] measured 4+4 whereas Hobbs *et al*. (1990) [14], Ahmadi (2004) [2], Ahmadi and Dalimi (2006) [3] and Thompson *et al*. (2006) [28] measured 3+ 3, while Sweatman and Williams (1963) [25] measured 5+5 arrangements from each rostellum.

In our study, 5 large and 5 small hooks were measured consistent with rostellum, which is believed to be a fairly large enough sample length to decrease the significance of outrageous values on the calculated sample mean. Correlation of the morphometric information acquired in the present examination with that from precursor reports uncovered that buffalo isolates are morphologically distinct and ought to represent a distinct strain.

It can be concluded that larval rostellar hook morphology can be used as a valid parameter for characterization of *E. granulosus* isolates. The data generated regarding the morphological characters of larval hooks could be used as the baseline values for brief identification of parasite in epidemiological studies.

Acknowledgement

The authors are highly thankful to the Department of Veterinary Parasitology, College of Veterinary and Animal Sciences and Govind Ballabh Pant University of Agriculture and Technology, Pantnagar for providing the Laboratory and Library facility.

References

1. Ahmad G, Nizami WA, Saifullah MK. Analysis of potential antigens of protoscolices isolated from pulmonary and hepatic hydatid cysts from *Bubalus bubalis*. Comp. Immunol. Microbiol. Infectious. Dis. 2001; 24:91-101.
2. Ahmadi N. Using morphometery of the larval rostellar hook to distinguish Iranian strains of *Echinococcus granulosus*. Ann. Trop. Med. Parasitol. 2004; 98:211-220.
3. Ahmadi N, Dalimi A. Characterization of *Echinococcus granulosus* isolates from human, sheep and camel in Iran. Infect Genetics Evolut. 2006; 6:85-90.
4. Almeida FB, Silva RR, Neves RH, Romanii ELS, Sliva

RM. Intraspecific variation of *Echinococcus granulosus* in livestock from Peru. Vet. Parasitol. 2007; 143:50-58.
5. Andresiuk M V, Gordo FP, Bandera CC, Elissondo MC, Dopchiz M, Denegri G, *et al*. *Echinococcus granulosus*: biological comparison of cattle isolates from endemic regions of Argentina and Spain. Review Argenenia Microbiol. 2013; 41:218-225.
6. Bhattacharya D, Bera AK, Bera BC, Pan D, Das S K. Molecular appraisal of Indian animal isolates of *Echinococcus granulosus.* Indian J Med. Res. 2008; 127:383-387.
7. Carmena D, Cardona GA. *Echinococcosis* in wild carnivorous species: epidemiology, genotypic diversity, and implications for veterinary public health. Vet. Parasitol. 2014; 202:69-94.
8. Constantine CC, Thompson RCA, Jenkins DJ, Hobbs RP, Lymbery AJ. Morphological characterization of adult *Echinococcus granulosus* as a means of determining transmission patterns. J Parasitol. 1993; 79:57-61.
9. Craig PS, McManus DP, Lightowlers MW. Prevention and control of cystic echinococcosis. The Lancet Infectious Dis, 2007; 7:385- 394.
10. Cucher MA, Macchiaroli N, Baldi G, Camicia F, Prada L, Maldonado L, Avila HG, *et al*. Cystic echinococcosis in South America: systematic review of species and genotypes of *Echinococcus granulosus sensu lato* in humans and natural domestic hosts. Trop. Med. Int. Hlth. 2016; 21:166-175.
11. Eckert J, Gemmell MA, Meslin F, Pawłowski ZS, WHO/OIE manual on echinococcosis in humans and animals: A public health problem of global concern. World Organisation for Animal Health (Office International des Epizooties) and World Health Organisation, 2001, 1-250.
12. Gemmell MA, Roberts MG. Population dynamics in echinococcosis and cysticercosis. New York: Oxford University Press, 1998, 665-688.
13. Gholami S, Irshadullah M, Mobedi I. Rostellar hook morphology of larval *Echinococcus granulosus* isolates from Indian buffalo, Iranian sheep, cattle and camel. J Helminthol. 2018; 85:239-245.
14. Hobbs RP, Lymbery AJ, Thompson RCA. Rostellar hook morphology of *Echinococcus granulosus* (Batsch, 1786) from natural and experimental Australian hosts and its implications for strain recognition. Parasitolo. 1990; 101:273-281.
15. Ito A, Nakao M, Sako Y. Echinococcosis: serological detection of patients and molecular identification of parasites. Future Microbiol. 2007; 2:439-449.
16. Jenkins DJ, Roming T, Thompson RCA. Emergencere/re-emergence of *Echinococcus* spp.: a global update. Int. J Parasitol. 2005; 35:1205-1219.
17. Karimi A, Dianatpour R. Genotypic and phenotypic characterization of *Echinococcus granulosus* of Iran. Biotechnol. 2008; 7:757-762.
18. McManus D. Echinococcosis with particular reference to Southeast Asia. Advancement in Parasitol. 2010; 72:267-303.
19. Nakao M, McManus D, Schantz P, Craig P, Ito A. A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. Parasitol. 2007; 134:713-722.
20. National Institute of Communicable Diseases (NICD). Zoonotic diseases of public health importance. Emerging Infect Dis. 2005; 11:1842.
21. Pednekar RP, Gatne ML, Thompson RCA, Traub RJ. Molecular and morphological characterisation of *Echinococcus* from food producing animals in India. Vet. Parasitol. 2009; 165:58-65.
22. Ponce Gordo F, Cuesta Badera CDifferentiation of Spanish strains of *Echinococcus granulosus* using larval rostellar hook morphometry. Int. J Parasitol. 1997; 27:41-49.
23. Sharma M, Sehga R, Fomda AB, Malhotra A, Malla N. Molecular characterization of *Echinococcus granulosus* cysts in north Indian patients: identification of G1, G3, G5 and G6 genotypes. PLoS Negl. Trop. Dis. 2013; 7:e2262.
24. Singh BB, Sharma JK, Ghataka S, Sharma R, Bal MS, Tuli A, *et al*. Molecular epidemiology of echinococcosis from food producing animals in North India. Vet. Parasitol. 2012; 186:503-506.
25. Sweatman GK, Williams RJ. Comparative studies on the biology and morphology of *Echinococcus granulosus* from domestic livestock, moose and reindeer. Parasitol. 1963; 53:339-390.
26. Tashani OA, Zhang LH, Boufana B, Jegi A, McManus DP. Epidemiology and strain characteristics of *Echinococcus granulosus* in the Benghazi area of Eastern Libya. Ann. Trop. Med. Parasitol. 2002; 96:369-381.
27. Thompson RCA, McManus DP. Towards a taxonomic revision of the genus *Echinococcus*. Trends in Parasitol. 2002; 18:452-457.
28. Thompson RCA, Boxell AC, Ralston BJ, Constantine CC, Hobbs RP, Shury T, *et al*. Molecular and morphological characterization of *Echinococcus* in cervids from North America. Parasitol. 2006; 132:439-447.
29. Thompson RCA, Kumaratilake LM, Eckert J. Observations on *Echinococcus granulosus* of cattle origin in Switzerland. Int. J Parasitol. 1984; 14:283-291.
30. World Health Organisation (WHO). Echinococcosis. Fact Sheet No.377. World Health organization Geneva-Switzerland updated March 2017, http://www.who.int/ mediacentre/ factsheets/fs377/en/