Journal of Veterinary Science & Technology

Rahman et al., J Veterinar Sci Technolo 2014, 5:5
DOI: 10.4172/2157-7579.1000208

Review Article Open Access

A Revisit to the Infamous Zoonotic Echinococcosis: A Molecular Review

Wahab A. Rahman[1]*, Layla E. Elmajdoub[2], Mohd Nor SA[2] and Wajidi MF[3]

[1]School of Food Science and Technology, Universiti Malaysia Terengganu, 21300 Kuala Terengganu, Malaysia
[2]School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
[3]School of Distance Education, Universiti Sains Malaysia, 11800 Penang, Malaysia

Abstract

Through the past five decades, significant phenotypic and genetic variabilities have been recognized and identified in various strains of *Echinococcus granulosus* isolated from different regions. Studies have revealed that the different strains of *E. granulosus* consist of heterogenous groups of genetic variants, which may display variations in morphology, host specificity, development rate, pathogenicity and geographical distributions. Thus identification of strain types is very important in strategizing and implementing an *Echinococcus* control and management program.

Keywords: Zoonotic echinococcosis; Molecular

Molecular Characterization of *Echinococcus granulosus*

Taxonomic studies of *E. granulosus* strains have been carried out based on different analytical methods such as morphology, epidemiology, biochemistry and molecular genetics [1]. These methods have proven to be useful, especially when used collectively. Thus, when morphological and molecular methods are conducted in complement they could provide more accurate and reliable information regarding the type and range of variation of *E. granulosus* [2-4]. However in the case of diagnosis of *E. granulosus* in the final hosts, different methods are used based on DNA analysis using Polymerase Chain Reaction [5,6].

Based on molecular data, *E. granulosus* have been classified into several genotypes [7-11] namely:

a) G1, common sheep strain.

b) G2: Tasmania sheep strain.

c) G3: Buffalo strain.

d) G4: Horse strain.

e) G5: Cattle strain.

f) G6: Camel strain.

g) G7: Pig strain.

h) G8: Cervid strain.

i) G9: Human strain. (Poland)

j) G10: Fennoscandian cervid strain

In addition, several researchers have suggested a review of this genus based on phylogenetic findings, specifically to re-classify several genotypes into species [9,12]. The identification of genotypes has at least two important applications. Firstly, it supports progressive DNA vaccination using recombinant DNA technology [13]. Secondly, it plays an extremely important role in studies on vaccination resistance [14]. In the past decade, molecular methods have been utilized globally to identify the most common strain of *E. granulosus* isolates including in studies in Algeria, Tunisia, and Mauritania [15-17].

However, only limited studies have been conducted in Libya [1,18]. In fact, on the whole, not much information is available regarding the molecular characterization of *Echinococcus granulosus* in North Africa. According to Eckert et al. [19], epidemiologic studies from different Middle Eastern regions indicate that camel is an important intermediate host that spreads the infection to humans.

Genetic Variation in *Echinococcus granulosus*

According to Thompson and McManus [12,20], a single important biological characteristic of *E. granulosus* is that it is composed of a number of intraspecific distinct features or strains that manifest in considerable variation at the genetic level. The term strain is used to describe variants that differ from other groups of the same species in gene frequencies or DNA sequences, and in one or more characters of factual significance to the epidemiology and control of hydatidosis [21,22]. Several researches have noted that the wide intraspecific variation in *E. granulosus* may be linked to life cycle patterns, development rate, host specificity, transmission methods and pathology [12,20,21]. Such a situation has a significant influence on the design and development of diagnostic reagents and vaccines regarding the epidemiology and control of echinococcosis.

Research in molecular methods based mainly on mitochondrial DNA sequences has identified 9 different genetic types of *E. granulosus*. In addition, genotype 10 was recently identified as a strain present in reindeer and moose in northeastern Finland [9]. Usually, the molecular approach is used when it is difficult to differentiate at the morphological characteristics.

Molecular Methods Utilized for Genetic Assessment of *Echinococcus*

The genetic variation of *Echinococcus* has been extensively explored based on sequences from mitochondrial and nuclear genomes. In this regard, Polymerase Chain Reaction (PCR)-based methods are highly sensitive and at present widely used for *Echinococcus* identification targets, including discrimination of eggs. The following is a description

***Corresponding author:** Wahab A. Rahman, School of Food Science and Technology, Universiti Malaysia Terengganu, 21300 Kuala Terengganu, Malaysia, Tel: +609 6684995; E-mail: arawahab@umt.edu.my

Received October 17, 2014; **Accepted** November 26, 2014; **Published** November 28, 2014

Citation: Rahman WA, Elmajdoub LE, Mohd Nor SA, Wajidi MF (2014) A Revisit to the Infamous Zoonotic Echinococcosis: A Molecular Review. J Veterinar Sci Technol 5: 208. doi:10.4172/2157-7579.1000208

of the various molecular methods used to study genetic variation in *Echinococcus* [23,24].

PCR-amplified DNA sequences

This method is based on a direct comparison of the nucleotide sequences between organisms and provides a highly reliable and sensitive diagnosis. The fragments of the mitochondrial genes cytochrome c oxidase subunit 1 (cox1), and the NADH dehydrogenase subunit 1 gene (nad1) have proven to be very useful in *E. granulosus* strain identification [23]. Dinkel et al. [25] developed rapid diagnostic approach by using the specific and sensitive semi-nested PCR system for *E. granulosus* genotypes G1 and G6/7 and *E. ortleppi* G5 genotype. The diagnosis of G1, G6 and G7 was accomplished by a simple PCR, whereas the differentiation between G5 and G6/7 included a subsequent semi-nested PCR step. In addition, the mitochondrial 12S ribosomal RNA gene was evaluated on isolates of 16 species of cestodes including *E. equinus* G4 and *E. ortleppi* G1, G5, G6 and G7 genotype. Saad and Magzoub [26] and Elmahdi et al. [27] revealed for the first time a camel strain G6 infection in humans in eastern Africa and cattle strain G5 in livestock from Sudan and Kenya respectively based on the PCR system.

Daniel Mwambete, et al. [28] tested genotype isolates of *E. granulosus* from different intermediate hosts based on the RAPD- PCR analysis in Spanish strains. Three strains, namely sheep-dog, horse-dog, and pig-dog of *E. granulosus* had been previously identified in Spain [28]. Daniel Mwambete, et al. [28] confirmed that the sheep strain G1 corresponded with genotype 1 but also infected sheep, goats, pigs, cattle and human. In addition they also confirmed that the horse strain corresponded to genotype 4 and only infected horse while the pig strain corresponded with genotype 7 and infected pigs and goats.

RFLP/RAPD analysis

Early DNA studies of genetic variation in *Echinococcus* had been focused on Restriction Fragment Length Polymorphism (RFLP) analysis based on the Southern blotting method [29-31]. In addition, Bowles and McManus [32] observed that the then current (RFLP) analysis was a simple process through binding rDNA RFLP analysis with PCR with no loss of solution or precision.

Azab, et al. [33] stated that previous studies of genetic variation which focused on RFLP analysis based on conventional southern blotting were able to differentiate between several strains of *E. granulosus*, were stable during analysis of a particular strain. The utility of Random Amplified Polymorphic DNA (RAPD-PCR) analysis has also been highlighted [34], when conducted under carefully controlled conditions. The method successfully characterized the four recognized *Echinococcus* species and different strains of *E. granulosus*. Furthermore, this method has been used in Egypt to identify the camel-dog strain [33].

Microsatellite markers

Microsatellites are short segments of DNA which have a repeated sequence and tend to occur in non-coding DNA. In comparison to the other methods, microsatellite DNA analysis is still underutilized in studying diversity in *Echinococcus* and only a few microsatellite markers are available for *E. multilocularis*. Microsatellite DNA was used for the first time by Bretagne et al. [35] to successfully assign *E. multilocularis* isolates into three groups. In addition, Nakao et al. [36] identified two microsatellite loci to demonstrate population level polymorphisms in *E. multilocularis* adults from wild foxes. In another study, Bartholomei-Santos, et al. [37] used eight oligonucleotides, including specific repeats as probes to characterize for the first time microsatellites of *E. granulosus* from Brazil and Argentina.

Classification of *Echinococcus granulosus* Strains

Sheep-dog (G1) and horse-dog (G4) strains

In their studies, McManus and Simpson [29]; McManus et al. [30] and McManus and Rishi [31] demonstrated the presence of sheep-dog and horse-dog strains in United Kingdom. Their findings were corroborated by Wachira et al. [38] who also detected the host predilection of sheep strain from Kenya while in Australia; Hope, et al. [39] demonstrated the presence of a single sheep genotypic strain in livestock animals.

In addition, it has been found that there are different strains of sheep-dog and horse-dog forms of *E. granulosus* which vary greatly in terms of biological criteria. For instance, Le, et al. [40] found that the sheep strain infects humans, whereas it may not be infective to horses. On the other hand, the horse strain appeared to be poorly infective to sheep as well as humans. Presently, based on DNA data, the sheep strain and horse strain differ by 12.4% in nucleotides and 11.6% in amino acids. Bowles, et al. [22] demonstrated an alternative process to detect levels of divergence based on the phylogenetic tree by using phylogenetic analysis of the sequences of mitochondrial and nuclear data. In another study, Le, et al. [40] used phylogenetic analysis to detect the level of divergence based on the phylogenetic tree of concatenated nad 1 and ATP6 genes from *E. multilocularis*, *E. oligarthrus*, *E. vogeli* and five genotypes from *E. granulosus* as illustrated in Figure 1.

Cattle-dog strain (G5)

According to McManus [41], up to the early 1990s, all human samples of *E. granulosus* examined by isoenzyme and DNA analysis belonged to the common sheep strain G1. However, the calcified hydatid cyst which was removed from young Dutch men and analyzed by PCR/RFLP test and cox1 and nad1 sequences, belonged to cattle strain G5, a strain found in Argentina [42,43]. Normally, cattle are the common host of hydatid cysts worldwide even though the sheep strain is more prevalent than the cattle strain. Moreover, according to Bowles, et al. [22], when cattle are infected by the sheep strain, the cattle is considered as an accidental host and the resultant cyst is usually infertile.

Although the molecular data for the cattle strain is not as rich

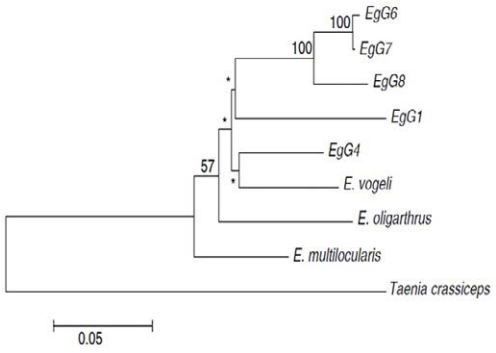

Figure 1: Inferred Relationships between Species and Genotypes of *Echinococcus*, 100% similarity between EgG6 and EgG7 also between EgG8, but between other species of *Echinococcus* the similarity 57% Using Concatenated Sequences of the Mitochondrial Genes ATP6, nad1 and cox1 Eg = *E. granulosus* = [40].

compared to the sheep strain, nevertheless the cattle strain has a widespread geographical distribution, including South Africa, India, Central Europe and South America [12]. Recently, Obwaller, et al. [10] revealed that the cattle strain G5 was shown by nad1 and cox1 sequences to infect Namibian zebra. However, only a limited number of molecular studies have been conducted on *Echinococcus* isolates from South African hosts to confirm the prevalence of this strain [41].

Camel-dog strain (G6)

The camel strain, which has been identified by DNA analysis, infects camels, cattle, goats and pigs in East Africa. It is also found in other countries including Iran, Argentina and China [41]. DNA studies have revealed that the camel strain infects camels, cattle and humans. In addition most of the studies from Mauritania, Egypt, and Iran have revealed the presence of the camel strain G6 in humans [15]. A recent study also revealed for the first time its presence in Kenyan human populations [25]. It was suggested that the camel strain has a shorter maturation time in dogs compared to the common sheep strain, hence more infective in humans.

Pig-dog strain (G7)

According to Scott, et al. [8], analysis of *Echinococcus* isolates from Poland indicated that the infection was not caused by the common sheep strain, G1 but the pig strain G7 as revealed by DNA analysis which showed very clear differences. However, DNA studies of pig and human isolates from Poland and Slovakia have failed to confirm the presence of this genotype, but have provided evidence for the almost exclusive existence of G7 [44,45].

Cervid strains (G8-10)

In North America and North Eurasia, the life cycle of the *E. granulosus* includes an intermediate moose and reindeer hosts with wolves and sledge dogs as the definitive hosts [46]. In Alaska, based on a single nad1 sequence and ITS PCR-RFLP pattern, the cervid strain, which obtained the G8 genotype in the moose [7]. However, Thompson and Lymbery [46] demonstrated that *E. granulosus* of cervid origin G8 differs in a number of biological assessments from domestic strains of *E. granulosus*.

Lavikainen, et al. [9] came up with a molecular guide to identify the presence of a new, distinct cervid strain. They observed that isolates of *E. granulosus* from reindeer and moose in Finland analyzed for mitochondrial cox1 and rDNA ITS-1 genes were similar, but high sequence variation was found in the ITS-1 region. However, the mitochondrial and nuclear sequences of the cervid strain from Finland and camel strain G6 closely resembled each other. The phylogenetic analysis (Figure 2) indicated that the Finnish isolates presumed to be G8 had a close relationship with G6 and G7.

The cervid strain initially assigned G8, appeared to represent a distinct, previously undescribed genotype of *E. granulosus* and was then reclassified by Lavikainen, et al. [9] as the Fennoscandian cervid strain G10. According to McManus [41], it is important to evaluate the geographical distribution of this new genotype in order to determine whether it is infective to humans as demonstrated in the case of G8 strain.

Based on mitochondrial genes (nad1, cox1), Le, et al. [40] in China showed that G1, G4, *E. vogeli*, and *E. oligarthrus* are almost equidistant from each other and the G1, G4 genotypes are nearly equidistant from G6, G8 genotypes (Figure 2). However G6, G7 and G8 are closely related and most likely belong to the same origin (livestock and human).

Molecular Phylogenetic Analysis

Studies conducted before the 1990s failed to show the close phylogenetic relationships among *Echinococcus* strains based on morphological and incomprehensive biochemical data. However, through molecular analysis, Bowles, et al. [22] recorded many characters, in particular, DNA sequence analyses which were very useful in the refining of the available morphological data based on phylogenies. In this regard, sequence data, including three nucleotide data sets, two mitochondrial (cox1; nad1) and one nuclear (ITS1) were used to elucidate the phylogenetic relationships of the *Echinococcus* strains. The combined mitochondrial data (Figure 3) and the nuclear data (Figure 4) revealed at least three evolutionarily discrete groups of *E. granulosus*. Furthermore, the molecular distances between them was comparable to or greater than the molecular evolutionary distances observed among recognized species suggesting that they were distinct taxa.

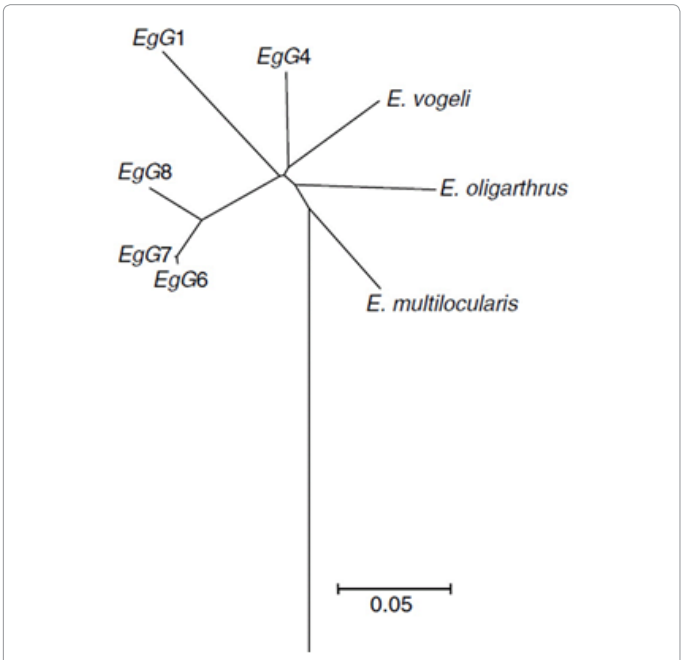

Figure 2: Relationships of G1, G4, G6, G7, G8, *E. vogeli*, and *E. oligarthrus* based on mtDNA, that EgG6, EgG7 and EgG8 with same branch [40].

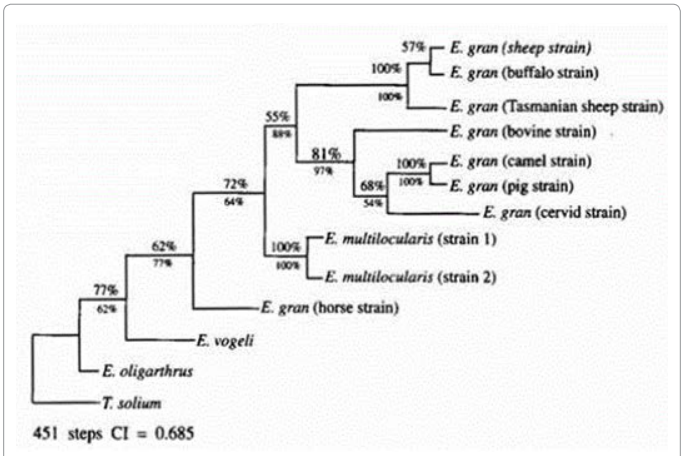

Figure 3: Phylogenetic relationships among *Echinococcus* species based on the mitochondrial (cox1; nad1) genes [22].

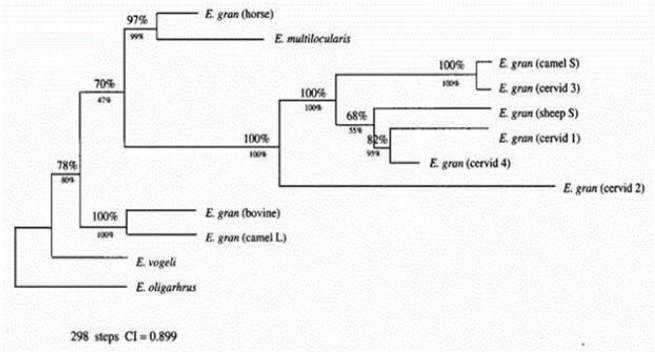

Figure 4: Phylogenetic relationships among *Echinococcus* species based on Nuclear Gene (ITS1) [22].

DNA Detection of Infection in Definitive and Intermediate Hosts Based on Enzyme-linked Immunosorbent Assay (ELISA) and Copro-DNA by PCR

Two methods have been successfully identified for the diagnosis of adult worms of *Echinococcus* in small intestines of definitive hosts [41]. The first is the investigation of *E. granulosus* specific coproantigens in Enzyme-Linked Immunosorbent Assay (ELISA) and the second is copro-DNA by PCR [47-52]. In addition to this application, these methods provide the means to study the transmission biology of *E. granulosus* as they allow investigation of infection in faecal samples collected from the environment.

According to Deplazes, et al. [48] the coproantigens (copro-PCR) method provides a sensitive, fast and cheap diagnosis compared to the PCR method alone, which is time consuming and expensive. In addition, the copro-PCR (antigens) is a useful method to confirm the positive coporantigen results based on ELISA, as taeniid eggs are difficult to differentiate morphologically. Therefore, the PCR (coproantigens) is the best method to use for identification of *Echinococcus* eggs in faecal samples, Dinkel, et al. [52].

Molecular Methods in Epidemiological Studies of Hydatid Cysts

On the other hand, Dinkel, et al. [52] described the routine PCR used in epidemiological studies and surveys of prevalence of hydatid cysts, especially small, atypical and calcified ones in intermediate hosts from different infected organs. In addition, Xiao, et al. [53] and Heath, et al. [54] discussed how PCR sequencing of mtDNA sequences have been utilized to reveal that Chinese yaks are unlikely to be infected by the sheep-dog strain of *E. granulosus* and *E. multilocularis*, although these two species of *Echinococcus* are common in China.

Hence, nucleic acid based methods and microsatellite markers are very useful in the investigation of genetic variation. In the last decade data on population and epidemiology genetics of *Echinococcus* strains has been rapidly accumulating and frequently, valuable information on the molecular categorization of genotypes is available. For instance, molecular techniques have been used to validate the genetic basis of important morphological and other biological differences [41]. These techniques provide a very reliable and simple way of addressing *Echinococcus* taxonomy and systematics. Indeed, investigations by Le, et al. [40]; Nakao, et al. [36] and Thompson and McManus [12] have provided genetic information that can be used for even more in-depth strain characterization and phylogeny construction of *Echinococcus* spp, The availability of a fairly comprehensive genetic database have provided a solid molecular basis for studying the taxonomy of the genus *Echinococcus*.

References

1. Tashani OA, Zhang LH, Boufana B, Jegi A, McManus DP (2002) Epidemiology and strain characteristics of Echinococcus granulosus in the Benghazi area of eastern Libya. Ann Trop Med Parasitol 96: 369-381.
2. McManus DP, Bryant C (1995) Biochemistry, physiology and molecular biology of Echinococcus. In: Thompson RCA, Lymbery AJ (Eds.), Echinococcus and Hydatid Disease. CAB International, Walingford, Oxon, UK, pp. 135-181.
3. McManus DP, Bowles J (1996) Molecular genetic approaches to parasite identification: Their value in diagnostic parasitology and systematics. Int J Parasitol 26: 687-704.
4. Eckert J, Thompson RC (1997) Intraspecific variation of Echinococcus granulosus and related species with emphasis on their infectivity to humans. Acta Trop 64: 19-34.
5. Mathis A, Deplazes P, Eckert J (1996) An improved test system for PCR-based specific detection of Echinococcus multilocularis eggs. J Helminthol 70: 219-222.
6. Trachsel D, Deplazes P, Mathis A (2007) Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology 134: 911-920.
7. Bowles J, Blair D, McManus DP (1994) Molecular genetic characterization of the cervid strain ('northern form') of Echinococcus granulosus. Parasitology 109 : 215-221.
8. Scott JC, Stefaniak J, Pawlowski ZS, McManus DP (1997) Molecular genetic analysis of human cystic hydatid cases from Poland: Identification of a new genotypic group (G9) of Echinococcus granulosus. Parasitology 114: 37-43.
9. Lavikainen A, Lehtinen MJ, Meri T, Hirvelä-Koski V, Meri S (2003) Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of Echinococcus granulosus. Parasitology 127: 207-215.
10. Obwaller A, Schneider R, Walochnik J, Gollackner B, Deutz A, et al. (2004) Echinococcus granulosus strain differentiation based on sequence heterogeneity in mitochondrial genes of cytochrome c oxidase-1 and NADH dehydrogenase-1. Parasitology 128: 569-575.
11. Magambo J, Njoroge E, Zeyhle E (2006) Epidemiology and control of echinococcosis in sub-Saharan Africa. Parasitol Int 55 Suppl: S193-195.
12. Thompson RC, McManus DP (2002) Towards a taxonomic revision of the genus Echinococcus. Trends Parasitol 18: 452-457.
13. Lodish H, Berk A, Zipursky LS, Matsudaira P, Baltimore D, et al. (2000) Recombinant DNA and genomics. In: "Molecular cell biology", 4th edition. Lodish H, Berk A, Zipursky LS, Matsudaira P, Baltimore D, Darnell J (eds). W.H. and Freeman Company, New York.
14. Amini-Bavil-Olyaee S, Alavian SM, Adeli A, Sarrami-Forooshani R, Sabahi F, et al. (2006) Hepatitis B virus genotyping, core promoter, and precore/core mutations among Afghan patients infected with hepatitis B: A preliminary report. J Med Virol 78: 358-364.
15. Bardonnet K, Piarroux R, Dia L, Schneegans F, Beurdeley A, et al. (2002) Combined eco-epidemiological and molecular biology approaches to assess Echinococcus granulosus transmission to humans in Mauritania: Occurence of the 'camel' strain and human cystic echinococcosis. Trans R Soc Trop Med Hyg 96: 383-386.
16. Bardonnet K, Benchikh-Elfegoun MC, Bart JM, Harraga S, Hannache N, et al. (2003) Cystic echinococcosis in Algeria: Cattle act as reservoirs of a sheep strain and may contribute to human contamination. Vet Parasitol 116: 35-44.
17. M'rad S, Filisetti D, Oudni M, Mekki M, Belguith M, et al. (2005) Molecular evidence of ovine (G1) and camel (G6) strains of Echinococcus granulosus in Tunisia and putative role of cattle in human contamination. Vet Parasitol 129: 267-272.
18. Abushhewa MH, Abushhiwa MH, Nolan MJ, Jex AR, Campbell BE, et al. (2011) Genetic classification of Echinococcus granulosus cysts from humans, cattle and camels in Libya using mutation scanning-based analysis of mitochondrial loci. Mol Cell Probes 24: 346-351.

19. Eckert J, Thompson RC, Michael SA, Kumaratilake LM, el-Sawah HM (1989) Echinococcus granulosus of camel origin: development in dogs and parasite morphology. Parasitol Res 75: 536-544.

20. Thompson RCA, McManus DP (2001) Aetiology: parasites and life cycles. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS (eds) WHO/ OIE Manual on Echinococcosis in humans and animals a public health problem of global concern. World Organization for Animal Health, Paris, pp: 1-19.

21. Thompson RC, Lymbery AJ (1990) Echinococcus: biology and strain variation. Int J Parasitol 20: 457-470.

22. Bowles J, Blair D, McManus DP (1995) A molecular phylogeny of the genus Echinococcus. Parasitology 110: 317-328.

23. McManus DP (2002) The molecular epidemiology of Echinococcus granulosus and cystic hydatid disease. Trans R Soc Trop Med Hyg 96 Suppl 1: S151-157.

24. McManus DP, Thompson RC (2003) Molecular epidemiology of cystic echinococcosis. Parasitology 127 Suppl: S37-51.

25. Dinkel A, Njoroge EM, Zimmermann A, Wälz M, Zeyhle E, et al. (2004) A PCR system for detection of species and genotypes of the Echinococcus granulosus-complex, with reference to the epidemiological situation in eastern Africa. Int J Parasitol 34: 645-653.

26. Saad JC, Magzoub M (1989) Hydatidosis in sheep and goats in the Sudan. Sudan Journal of Veterinary Science and Animal Husbandary, 28: 33-37.

27. Elmahdi IE, Ali QM, Magzoub MM, Ibrahim AM, Saad MB, et al. (2004) Cystic echinococcosis of livestock and humans in central Sudan. Ann Trop Med Parasitol 98: 473-479.

28. Daniel Mwambete K, Ponce-Gordo F, Cuesta-Bandera C (2004) Genetic identification and host range of the Spanish strains of Echinococcus granulosus. Acta Trop 91: 87-93.

29. McManus DP, Simpson AJ (1985) Identification of the Echinococcus (hydatid disease) organisms using cloned DNA markers. Mol Biochem Parasitol 17: 171-178.

30. McManus DP, Simpson AJG, Rishi AK (1987) Characterization of the hydatid disease organism Echinococcus granulosus from Kenya using cloned DNA markers. In: Geerts S, Kumar V, Brandt J, eds, Helminth Zoonoses, pp: 29-36.

31. McManus DP, Rishi AK (1989) Genetic heterogeneity within Echinococcus granulosus: isolates from different hosts and geographical areas characterized with DNA probes. Parasitology 99: 17-29.

32. Bowles J, McManus DP (1993) Molecular variation in Echinococcus. Acta Trop 53: 291-305.

33. Azab ME, Bishara SA, Helmy H, Oteifa NM, El-Hoseiny LM, et al. (2004) Molecular characterization of Egyptian human and anima Echinococcus granulosus isolates by RAPD-PCR technique. J Egypt Soc Parasitol 34: 83-96.

34. Scott JC, McManus DP (1994) The random amplification of polymorphic DNA can discriminate species and strains of Echinococcus. Trop Med Parasitol 45: 1-4.

35. Bretagne S, Assouline B, Vidaud D, Houin R, Vidaud M (1996) Echinococcus multilocularis: microsatellite polymorphism in U1 snRNA genes. Exp Parasitol 82: 324-328.

36. Nakao M, Sako Y, Ito A (2003) Isolation of polymorphic microsatellite loci from the tapeworm Echinococcus multilocularis. Infect Genet Evol 3: 159-163.

37. Bartholomei-Santos ML, Heinzelmann LS, Oliveira RP, Chemale G, Gutierrez AM, et al. (2003) Isolation and characterization of microsatellites from the tapeworm Echinococcus granulosus. Parasitology 126: 599-605.

38. Wachira TM, Bowles J, Zeyhle E, McManus DP (1993) Molecular examination of the sympatry and distribution of sheep and camel strains of Echinococcus granulosus in Kenya. Am J Trop Med Hyg 48: 473-479.

39. Hope M, Bowles J, McManus DP (1991) A reconsideration of the Echinococcus granulosus strain situation in Australia following RFLP analysis of cystic material. Int J Parasitol 21: 471-475.

40. Le TH, Pearson MS, Blair D, Dai N, Zhang LH, et al. (2002) Complete mitochondrial genomes confirm the distinctiveness of the horse-dog and sheep-dog strains of Echinococcus granulosus. Parasitology 124: 97-112.

41. McManus DP (2006) Molecular discrimination of taeniid cestodes. Parasitol Int 55 Suppl: S31-37.

42. Kamenetzky L, Gutierrez AM, Canova SG, Haag KL, Guarnera EA, et al. (2002) Several strains of Echinococcus granulosus infect livestock and humans in Argentina. Infect Genet Evol 2: 129-136.

43. Haag KL, Ayala FJ, Kamenetzky L, Gutierrez AM, Rosenzvit M (2004) Livestock trade history, geography, and parasite strains: The mitochondrial genetic structure of Echinococcus granulosus in Argentina. J Parasitol 90: 234-239.

44. Kedra AH, Swiderski Z, Tkach VV, Dubinsky P, Pawlowski Z, et al. (1999) Genetic analysis of Echinococcus granulosus from humans and pigs in Poland, Slovakia and Ukraine. A multicenter study. Acta Parasitologica 44: 248-254.

45. Pawłowski Z, Stefaniak J (2003) The pig strain of Echinococcus granulosus in humans: a neglected issue? Trends Parasitol 19: 439.

46. Thompson RC, Lymbery AJ (1988) The nature, extent and significance of variation within the genus Echinococcus. Adv Parasitol 27: 209-258.

47. Stefanić S, Shaikenov BS, Deplazes P, Dinkel A, Torgerson PR, et al. (2004) Polymerase chain reaction for detection of patent infections of Echinococcus granulosus ("sheep strain") in naturally infected dogs. Parasitol Res 92: 347-351.

48. Deplazes P, Dinkel A, Mathis A (2003) Molecular tools for studies on the transmission biology of Echinococcus multilocularis. Parasitology 127 Suppl: S53-61.

49. Abbasi I, Branzburg A, Campos-Ponce M, Abdel Hafez SK, Raoul F, et al. (2003) Copro-diagnosis of Echinococcus granulosus infection in dogs by amplification of a newly identified repeated DNA sequence. Am J Trop Med Hyg 69: 324-330.

50. Mathis A, Deplazes P (2002) Role of PCR-DNA detection of Echinococcus multilocularis. In: Craig P, Pawlowski Z (eds) Cestode Zoonoses: Echinococcus and Cysticercosis. IOS Press, Amsterdam, pp. 195-204.

51. Cabrera M, Canova S, Rosenzvit M, Guarnera E (2002) Identification of Echinococcus granulosus eggs. Diagn Microbiol Infect Dis 44: 29-34.

52. Dinkel A, von Nickisch-Rosenegk M, Bilger B, Merli M, Lucius R, et al. (1998) Detection of Echinococcus multilocularis in the definitive host: coprodiagnosis by PCR as an alternative to necropsy. J Clin Microbiol 36: 1871-1876.

53. Xiao N, Qiu J, Nakao M, Nakaya K, Yamasaki H, et al. (2003) Short report: Identification of Echinococcus species from a yak in the Qinghai-Tibet plateau region of China. Am J Trop Med Hyg 69: 445-446.

54. Heath DD, Zhang LH, McManus DP (2005) Short report: Inadequacy of yaks as hosts for the sheep dog strain of Echinococcus granulosus or for E. Multilocularis. Am J Trop Med Hyg 72: 289-290.