Parasitol Res (2007) 100:495–503
DOI 10.1007/s00436-006-0286-9

ORIGINAL PAPER

Taxonomic position and geographical distribution of the common sheep G1 and camel G6 strains of *Echinococcus granulosus* in three African countries

S. Maillard · M. C. Benchikh-Elfegoun · J. Knapp · J. M. Bart · P. Koskei · B. Gottstein · R. Piarroux

Received: 12 May 2006 / Accepted: 11 July 2006 / Published online: 3 October 2006

Abstract The taxonomic and phylogenetic status of *Echinococcus granulosus* strains are still controversial and under discussion. In the present study, we investigated the genetic polymorphism of *E. granulosus* isolates originating from three countries of Africa, including a region of Algeria, where the common G1 sheep and the camel G6 strains coexist sympatrically. Seventy-one hydatid cysts were collected from sheep, cattle, camels, and humans. Two mitochondrial markers (*cox1* and *nad1*) were used for strain identification. Two nuclear markers (*actII* and *hbx2*) were used to study the possible occurrence of cross-fertilization. Despite the heterogeneity observed among the G1 isolates, they were all localized within one robust cluster. A second strong cluster was also observed containing all of the G6 isolates. Both strains appeared as two distinct groups, and no cases of interbreeding were found. Thus, the attribution of a species rank can be suggested. We also found the Tasmanian sheep G2 strain for the first time in Africa. Because of the slight variations observed between the common sheep and the Tasmanian sheep strains, further studies should be carried out to elucidate the epidemiological relevance of this genetic discrimination.

S. Maillard · J. Knapp · J. M. Bart · R. Piarroux
SERF ("Santé Environnement Rural-Université de Franche-Comté"),
Parasitology Department, University of Franche-Comté,
Besancon, France

M. C. Benchikh-Elfegoun
Laboratory of Parasitology, Veterinary Department,
University of Mentouri,
Constantine, Algeria

P. Koskei
Faculty of Veterinary Medicine,
Addis Ababa University,
Debre Zeit, Ethiopia

B. Gottstein
Institute of Parasitology, University of Bern,
Bern, Switzerland

S. Maillard (✉)
Laboratoire de Parasitologie, Equipe de recherche SERF,
Faculté de Médecine-Pharmacie,
19 Rue Ambroise Paré,
25000 Besançon, France
e-mail: maillard_steph@yahoo.com

Introduction

Cystic echinococcosis (CE), caused by the larval infection with the Cestoda *Echinococcus granulosus,* is a highly endemic zoonosis in the ruminant breeding areas of North Africa. The parasite is mainly transmitted in a synanthropic cycle generally involving dogs (large stray populations) and livestock animals (sheep, cattle, goats, and camels; Thompson and McManus 2001). DNA-based studies have shown that the species *E. granulosus* is composed of heterogeneous groups of genetic variants, defined as "strains" (McManus 2002). In various studies, strains were often characterized after polymerase chain reaction (PCR) amplification by sequencing mitochondrial markers in *cytochrome c oxydase 1* and *nicotinamide adenine dinucleotide* (*reduced form*; *NADH*) *dehydrogenase 1* genes. Each identified haplotype, ranging from genotypes 1 to 10, was associated with particular host specificity and epidemiological patterns (Bowles and McManus 1993; Bowles et al. 1992; Thompson et al. 1995; Eckert and Thompson 1997). For example, the common sheep G1 strain is widespread and has been mainly identified in Mediterranean and semiarid breeding areas as being carried by sheep and cattle. In contrast, the

camel G6 strain is often found in desert areas, carried by camels. Most human infections are due to the common sheep strain, whereas only a few cases have been attributed to the camel strain. For some authors, the definition of all the *E. granulosus* strains should be reassessed (Obwaller et al. 2004; Thompson et al. 2005). It has not yet been demonstrated that each strain forms a true clade (i.e. a monophyletic taxon) with a real epidemiological relevance. According to Tassy (1986), a taxon is a group of organisms recognized as a unit at each level of classification. Besides the differences in intermediate host specificity, geographic distribution, and genetic profiles, reproduction processes are also of importance in cladistics (Mayr et al. 1953). Up to now, *E. granulosus* strains have been mainly investigated by sequencing mitochondrial markers. Because of the asexual transmission (matrilineal origin) of the mitochondrial genes and the nonreticulated trait of the mtDNA molecules (Avise and Walker 1999), this method cannot be used for investigation of heterozygote patterns which evoke cross-fertilization.

In North and East Africa, two strains, the common sheep and camel, are widespread (Sadjjadi 2005). The sheep strain, the most infective for humans, has been described in Morroco and northern Algeria (Bart et al. 2004), in Kenya (Wachira et al. 1993; Dinkel et al. 2004), in Sudan (Dinkel et al. 2004), in Tunisia (Tashani et al. 2002; Lahmar et al. 2004; M'Rad et al. 2005), in Egypt (Macpherson and Wachira 1997), and in Libya (Tashani et al. 2002). In these studies, the sheep strain has been found in sheep, cattle, goats, pigs, and more rarely, in camels (some cases in Algeria, Kenya, Libya, and Tunisia). The camel strain has not often been identified in humans living in North Africa except for Egypt (Azab et al. 2004) and Mauritania (Bardonnet et al. 2002), where it has been presumed to play an important role in the transmission of CE to humans. According to the previously cited studies, this strain has been found in Mauritania, Tunisia, Egypt, Sudan, and also in nearby East Africa (Kenya) to be carried by livestock hosts such as camels and sometimes, sheep, cattle, and goats. On the northern border of the Sahara, including Algeria, Tunisia, and Egypt, both strains have been described in coexistence and sharing the same definitive host (i.e., dogs). The hypothesis of a strain concept should be tested by collecting samples in sympatric areas and searching for heterozygote patterns using nuclear DNA markers (Haag et al. 1999; Bartholomei-Santos et al. 2003; Nakao et al. 2003) to prove cross-fertilization.

In the present work, 71 *E. granulosus* samples (metacestode stage) were collected from slaughterhouses and human hospitals from three different countries in North Africa (Algeria, Mauritania, and Ethiopia). They were typed by two mitochondrial DNA markers [*cytochrome c oxydase 1* (*cox1*), and *NADH dehydrogenase 1* (*nad1*) genes] and two nuclear DNA markers [*actin II* (*actII*) and homeobox 2 (*hbx2*) genes]. Our first objective was to identify the geographical, biological, and genetic variants occurring in the regions. The second objective was to search for heterozygotes in the sympatrically populated "Touggourt-Ouargla" area of Algeria to document the possible occurrence of cross-fertilizing processes.

Materials and methods

Sampling strategy

Echinococcus granulosus whole hydatid cysts were collected from intermediate hosts either at slaughterhouses or in human hospitals in three countries of North Africa: Algeria, Mauritania, and Ethiopia (see characteristics in Table 1). The 46 Algerian samples were collected by local teams: 23 from the North (5 sheep and 13 cattle samples came from slaughterhouses in eight different cities, and 5 human samples came from hospitals in four different cities) and 23 from the more southern "Touggourt-Ouargla" region (ten sheep, three cattle, and ten camel samples). Algeria was of particular interest for this study due to the sympatric coexistence of both common sheep and camel strains in the Touggourt-Ouargla region (Bart et al. 2004). The two zones were also of interest because they contrasted with regard to climate, landscape morphology, human population density (which probably impacts on the dog population density), livestock herd composition and distribution, methods of breeding, and disease control guidelines. The 20 Mauritanian samples came from the Nouakchott region (1 cattle, 17 camel, and 2 human samples). Cattle breeding used to be Mauritania's largest commercial activity; during the last few decades, the livestock has been drastically reduced due to chronic drought and the rapid advance of the desert. These changes have brought many shifts in patterns of herd management (ownership, composition, and movements) and have increased the pressures on the land, also occupied by sedentary farmers. The successive droughts have also caused many changes in the methods used for the breeding of camels (herds traditionally located in the drier north) and for sheep (herds usually located throughout Mauritania). The capital, Nouakchott, was a major refugee center during the severe Saharan droughts of the 1970s and, thus, grew very quickly. The city became a large commercial hub and the center of large livestock migrations. The five Ethiopian samples were collected as part of a study carried out by the Institute of Parasitology of Bern (Switzerland) in the capital Addis Ababa (four sheep samples) and in a nearby city, Asela (one cattle sample). These sites were interesting because they are located in the Ethiopian highlands, where the highest concentration of livestock production can be

Table 1 Characteristics of the Algerian (a), Mauritanian (b), and Ethiopian (c) samples used in this study

Host	Origin	*cox1*	*nad1*	*actII*	*hbx2*	Mitochondrial genotypes	Nuclear genotypes
a.							
Cattle	Sétif (N)	DQ341564	DQ341515	DQ341539	DQ341554	m1	n1
	–	–	–	–	–	–	–
	Annaba (N)	–	–	–	–	–	–
	Batna (N)	–	–	–	–	–	–
	Jijel (N)	–	–	–	–	–	–
	Constantine (N)	–	–	–	–	–	–
	Ouargla (TOR)	–	–	–	–	–	–
		–	–	–	–	–	–
		–	–	–	–	–	–
	El Eulma (N)	DQ341570	–	–	–	m2	–
	Constantine (N)	DQ341571	DQ341520	–	–	m3	–
		–	–	–	DQ341558	–	n2
		–	DQ341521	–	–	m4	–
		DQ341572	–	–	–	m5	–
		DQ341573	DQ341522	–	–	m6	–
		DQ341573	–	–	–	m7	–
Sheep	Constantine (N)	DQ341566	DQ341517	DQ341541	DQ341552	m1	n1
	Touggourt (TOR)	–	–	–	–	–	–
	Ouargla (TOR)	–	–	–	–	–	–
		–	–	–	–	–	–
	Batna (N)	DQ341574	DQ341523	–	–	m8	–
	Sétif (N)	–	DQ341526	–	–	m9	–
	Aïn M'lila (N)	–	DQ341527	–	–	m10	–
		DQ341577	DQ341528	–	–	m11	–
	Ouargla (TOR)	DQ341577	DQ341528	–	–	m11	–
		DQ341577	DQ341528	–	–	m11	–
	Touggourt (TOR)	–	–	DQ341545	–	–	n3
		–	–	DQ341545	–	–	n3
	Ouargla (TOR)	–	–	–	DQ341559	–	n4
		DQ341579	–	–	–	m12	–
		–	DQ341530	–	–	m13	–
Dromedary	Touggourt (TOR)	DQ341581	DQ341531	DQ341549	DQ341561	m14	n5
	–	–	–	–	–	–	–
	–	–	DQ341535	–	–	m15	–
	Ouargla (TOR)	DQ341535	–	–	M15	–	–
		DQ341535	–	–	M15	–	–
	Touggourt (TOR)	–	–	DQ341546	–	m14	n6
	–	–	–	DQ341547	–	–	n7
	Ouargla (TOR)	–	DQ341536	–	–	m16	–
		DQ341575	DQ341524	DQ341543	DQ341557	m8	n1
		DQ341568	DQ341529	DQ341543	DQ341557	m17	n1
Human	Constantine (N)	DQ341569	DQ341519	DQ341544	DQ341556	m1	n1
	–	–	–	–	–	–	–
	Batna (N)	–	–	–	–	–	–
	Tébessa (N)	DQ341578	–	–	–	m18	–
	Jijel (N)	DQ341576	DQ341525	–	–	m8	–
b.							
Cattle	Nouakchott (M)	DQ341580	DQ341533	DQ341548	DQ341560	m14	n5
Dromedary	Nouakchott (M)	DQ341582	DQ341532	DQ341550	DQ341562	m14	n5
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–

Table 1 (continued)

Host	Origin	*cox1*	*nad1*	*actII*	*hbx2*	Mitochondrial genotypes	Nuclear genotypes
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
Human	Nouakchott (M)	DQ341583	DQ341534	DQ341551	DQ341563	m14	n5
	–	DQ341584	–	–	–	m19	–
c.							
Cattle	Assela (E)	DQ341565	DQ341516	DQ341540	DQ341555	m1	n1
Sheep	Addis Ababa (E)	DQ341567	DQ341518	DQ341542	DQ341553	m1	n1
	–	DQ341585	–	–	–	m20	–
	–	–	DQ341537	–	–	m21	–
	–	–	DQ341538	–	–	m22	–

The parasitic material extracted from them was characterized with two mitochondrial and two nuclear markers (*cox1*, *nad1*, *actII*, and *hbx2*). The GenBank accession numbers of the sequences from DQ341515 to DQ341585 are indicated (GenBank database, http://www.ncbi.nlm.nih.gov/entrez). Mitochondrial genotypes were defined from the two mitochondrial markers; nuclear genotypes were defined from the two nuclear markers. These genotypes were then used to draw dendograms based on the genetic distances between the sequences.

found. Up until now, only one study of molecular strain identification has been carried out in Mauritania and none in Ethiopia. The study in Mauritania showed that only the camel strain was circulating and, thus, was playing a major role in the transmission to humans (Bardonnet et al. 2002). Despite the lack of studies specific to Ethiopia, studies carried out in nearby East Africa suggest the presence of both common sheep and camel strains in this focus (Wachira et al. 1993; Dinkel et al. 2004).

Molecular and phylogenetic analysis

DNA extraction

The genomic DNA was extracted from hydatid tissue (protoscoleces and/or germinal layer) using a High Pure PCR Preparation Kit (Roche Diagnostics, Mennheim, Germany). For the fertile cysts (presence of protoscoleces), we carefully followed the manufacturer's instructions (digestion by proteinase K) except for the sterile ones, where the samples were prepared as follows: (1) enucleated cysts were "frozen–thawed" three times at −20/37°C to separate the germinal layer from the laminated layer, (2) the germinal layer was minced and broken in three consecutive baths of liquid nitrogen, and (3) the samples were incubated with lyzosyme for 1 h at 37°C and then incubated with sodium dodecyl sulfate (SDS), proteinase K, and lysis buffer overnight at 55°C.

DNA sequencing

The total DNA was subsequently used as a template for the amplification of two mitochondrial markers (*cox1*, a 309-bp portion of the gene coding for cytochrome *c* oxydase 1; and *nad1*, a 279-bp sequence of the gene coding for NADH dehydrogenase 1) and two nonrepeated nuclear markers (*actII*, an intronic 240-bp portion of the gene coding for actin II; and *hbx2*, a mainly intronic 257-bp sequence included in a homeobox gene). The PCR was performed in a 25-µl volume containing 2 µl genomic DNA, 100 µM each deoxynucleoside triphosphate (dNTP; MBI Fermentas, Vilnius, Lithuania), 20 pmol each of primers (Invitrogen Life Technologies, Paisley, Scotland) and 0.5 U DNA Red Taq Polymerase in 2.5 µl reaction buffer ×10 (Sigma, Saint Louis, MO, USA). The characteristics of the four pairs of primers used in the present work, the annealing temperatures they required for PCR, and the time chosen for elongation according to the length of markers are shown in Table 2. The amplification conditions were as follows: an initial step of denaturation (30 s at 94°C) followed by 40 cycles of successive denaturation (30 s at 94°C), hybridization (30 s at the annealing temperature), and elongation (30–75 s at 72°C), and then a final elongation of 5 min at 72°C. The specificity of amplications and the size of products were assessed by electrophoresis in 1.5% (w/v) Tris–acetate/EDTA (TAE) agarose gels. Using 1 µl of ExoSAP-IT (USB Corporation, Cleveland, OH, USA), 2.5-µl

Table 2 Primer sequences and PCR conditions for the amplification of the nuclear and mitochondrial markers

Targets		Primers	Length of the amplified fragment (bp)	Annealing temperature (°C)	Elongation time (s)	Reference
Nuclear						
actII	Forward	5′-gTCTTCCCCTCTATCgTggg-3′	266	60	75	Da Silva et al. (1993)
	Reverse	5′-CTAATgAAATTAgTgCTTTgTgCgC-3′				
hbx2	Forward	5′-TTCTCCTCTAgCCAggTCCA-3′	331	56	45	Haag et al. (1997)
	Reverse	5′-TATAgCgCCgATTCTggAAC-3′				
Mitochondrial						
cox1	Forward	5′-TTTTTTggCCATCCTgAggTTTAT-3′	396	60	75	Bart et al. (2006)
	Reverse	5′-TAACgACATAACATAATgAAAATg-3′				
nad1	Forward	5′-AgTCTCgTAAgggCCCTAACA-3′	488	45	30	
	Reverse	5′-CCCgCTgACCAACTCTCTTTC-3′				

PCR products were then purified for 15 min at 37°C and 15 min at 80°C. For sequencing, 2 µl distilled water, 1 pmol forward or reverse primer, and 2 µl DTCS Quick Start Master Mix (Beckman Coulter, Fullerton, CA, USA) were added. The reaction conditions were as follows: 40 cycles of 20 s at 94°C, 20 s at 50°C and 4 min at 60°C. After alcoholic purification, the migration of the fragments was performed in an automatic sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter).

Sequence analysis and phylogenetic reconstruction

The nucleotide sequences were subsequently examined by aligning them with the Bioedit software and its application ClustalW (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Using another Bioedit application, basic local alignment search tool (BLAST), the nucleotide sequences were also compared to those in the GenBank database (http://www.ncbi.nlm.nih.gov), which serve as the references for defining the *E. granulosus* common sheep (G1), Tasmanian sheep (G2), and camel (G6) strains, and *Echinococcus multilocularis* (GenBank references shown in Fig. 1). All these sequences, either obtained or referenced, were then used to draw dendograms using MEGA software (Molecular Evolutionary Genetics Analysis 3.1, (http://www.megasoftware.net). The neighbor-joining (NJ) method was chosen because its heuristic-clustering algorithm is based on the principle of minimum evolution, that is, the minimum number of changes which can occur between the sequences (Saitou and Nei 1987) and because it takes into account the possible differences in their speeds of occurrence. This method was corrected by the Kimura two-parameter model (Kimura 1980), which includes the equal base frequencies and the differences existing between the rates of transversion (substitution of a purine for a pyrimidine or vice versa) and transition (purine/purine or pyrimidine/pyrimidine substitution). To assess the robustness of the branches, a bootstrap test (Felsenstein 1985) of 1,000 repetitions was performed. Based on the statistical inference and using our matrix of data, this technique constructed a new set of sequences (lines) 1,000 times in randomly selected nucleotide positions (columns) without replacing them. A dendogram was drawn for each set with the NJ method. In the analysis of these 1,000 repetitions, the number of trees in which a genetic cluster appeared was associated with its robustness. A clustering of genotypes was considered relevant when its robustness was more than 70%.

Results

Sequence analysis

The sequences obtained by the characterization of the four DNA markers chosen for this study are shown in Table 1. A total of 34 different genotypes were identified in typing *cox1* (11), *nad1* (14), *actII* (5), and *hbx2* (4) markers. All the sequences are available on GenBank, with accession numbers from DQ341515 to DQ341585 (Table 1). The rate of polymorphism indicated by these markers (i.e., their power of discrimination) was higher for the mitochondrial targets than for the nuclear ones (22 different genotypes vs only 7, respectively). The detailed analysis of the 142 nuclear sequencing electrophoregrams did not reveal any patterns suggesting the occurrence of cross-fertilization (as did the G1/G5 SSCP profiles presented by Haag et al. 1999).

Mitochondrial markers

Among the mitochondrial sequences, we identified one set defined by a homology of 99.4 to 100% with G1 and a second one, defined by a homology of 89.1 to 89.4% with G1 and identified as a cluster of G6 sequences. All of the Algerian cattle, sheep, and human samples formed the G1

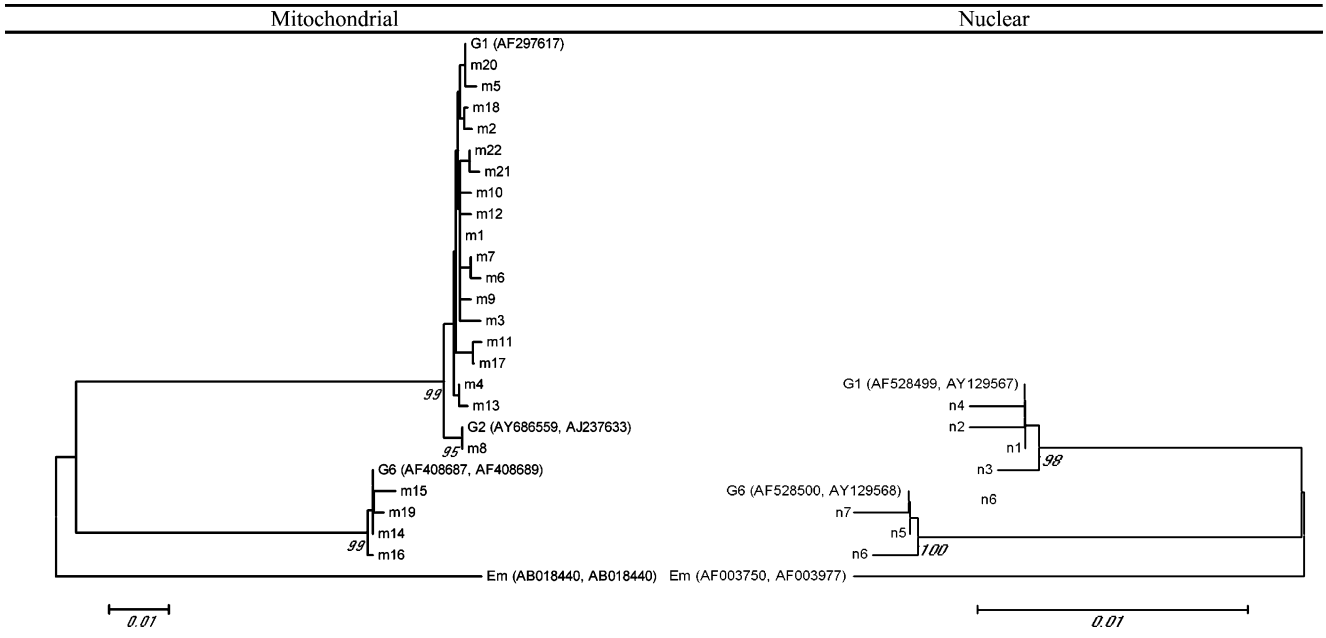

Fig. 1 Comparison of the two dendograms drawn with the mitochondrial and nuclear genotypes identified in this study. These genotypes were defined by combining the results obtained with the four different typing targets (*cox1*, *nad1*, *actII*, and *hbx2*). The phylogenetic trees were drawn using the neighbor-joining method and the Kimura two-parameter model of correction (MEGA software v. 3.1, http://www.megasoftware.net)

cluster, whereas most of the Algerian camel samples formed the G6 cluster. The coexistence of the common sheep and the camel strains was confirmed in the Touggourt-Ouargla region of Algeria. In addition, this sympatry was underlined by the identification of the common sheep strain in a camel from Ouargla, a host usually infected by the camel strain. The so-called "Tasmanian sheep" G2 strain was also found in our panel. Until now, no study has reported its presence in Africa. The G2 strain was identified in a human and a sheep from northern Algeria and in a camel from Ouargla. When investigating the potential polymorphism of all the 46 sequences identified in Algerian samples, a higher genetic variability was observed in the G1 cluster than in the G6 cluster. The three Algerian samples carrying the Tasmanian sheep strain showed the same G2 nucleotide sequence. The Mauritanian samples showed a high homogeneity because they all had the same G6 sequence; this particular sequence was also identified previously in four of eight Algerian G6 camels. In addition, our study also confirmed the presence of the common sheep G1 strain in Ethiopia: four different G1 sequences were identified in the five Ethiopian samples.

The clustering of sequences observed in Algeria was also observed in the Mauritanian and Ethiopian data. Indeed, despite the high heterogeneity of all the Algerian and Ethiopian G1 sequences found in the present work, they were all clustered in one set. In addition, the G6 cluster from the Algerian samples was not disturbed when the Mauritanian ones were added. In both genetic discrete units, some microvariants were characterized: we identified seven G1 and one G6 sequences for *cox1*, as well as nine G1 and two G6 sequences for *nad1*. Compared to the genotypes most frequently observed, these differed by 1 to 2 bp, and no one variant was found in more than four hosts.

Nuclear markers

Mitochondrial marker analyses were compared with those of the nuclear markers in which two clusters were also identified. The sequences of the first cluster exhibited a 99.7 to 100% identity with the combination of sequences described as G1 by Bart et al. (2004) from Algerian samples (rGB, AF528499 and AY129567), whereas the second exhibited a 99.3 to 99.5% identity with the same references and was linked with G6 sequences also described in the work just cited (rGB, AF528500 and AY129568). In contrast to the mitochondrial data, discrimination between the Tasmanian sheep and common sheep strains was not possible with the nuclear tools chosen for this study. For the nuclear markers, some microvariants were identified: one G1 and two G6 sequences for *actII*, and two G1 but no G6 sequences for *hbx2*. Compared with the genotypes most frequently identified, these microvariants differed by only 1 bp and were isolated from not more than two different samples.

Phylogenetic reconstruction

From the mitochondrial and nuclear data, two dendograms were drawn (Fig. 1), and each of them showed two genetic clusters, also named "discrete typing units" (DTUs) (Tibayrenc 2003), corresponding to the common sheep G1 and camel G6 strains of *E. granulosus*. Both units had high values in the bootstrap tests (from 98 to 100%), thus showing a high robustness. In all of the sequences obtained from our panel of samples, all G1 sequences formed the first DTU, whereas all the other G6 sequences formed the second DTU; this DTU distribution was the same in both mitochondrial and nuclear dendograms. With regard to the Tasmanian sheep strain, a distinct cluster formed by the G2 sequences had a high robustness (95%), but only in the mitochondrial dendogram. Furthermore, whereas the divergence observed between the G1 and G6 branches amounted to 0.013 and 0.004 for the mitochondrial and nuclear trees, respectively, the mitochondrial G2 DTU showed a genetic divergence from the G1 DTU equal to 0.003.

Discussion

In the present study, the molecular characterization of 71 *E. granulosus* samples by four DNA markers, selected from the mitochondrial and nuclear genomes of the parasite, led to the identification of 34 distinct genotypes. All of them were clearly clustered in two sets, one formed by all G6 samples and the other by all the G1 and G2 samples. None of these samples exhibited evidence of genetic exchanges between the two sets such as the specific heterozygote profiles previously described by Haag et al. (1999), Bartholomei-Santos et al. (2003), and Nakao et al. (2003). For the first time in Africa, we also showed the presence of genotype 2 attributed to the so-called Tasmanian sheep strain. It was found in sheep, human, and camel samples. Our results help to clarify the status of these strains within *E. granulosus* species. Although the coexistence of the common sheep and camel strains along the Algerian northern border of the Sahara, in agreement with the findings of Bart et al. (2004), and the occurrence of a host spectrum overlap, both strains were clearly distinct, and we found no case evoking a genetic exchange between the two. In the mitochondrial and nuclear dendograms, the strains were clustered in two discrete units, both showing a high level of robustness. Regarding the intermediate host spectrum, the two clusters generally corresponded to two distinct life cycles, that is, sheep/cattle/human vs camel. Finally, except for all the samples originating from the Touggourt-Ouargla region in Algeria, the two sets also corresponded to two distinct geographical distributions, a desert area (camel strain) and a more humid area (common sheep and Tasmanian sheep strains).

Thus, the sympatric coexistence of the common sheep and camel strains was confirmed for the Touggourt-Ouargla region of Algeria. Contrary to the evidence for two separate and distinct life cycles, we found two camels infected by the two sheep strains (i.e., one with the common sheep strain and the other one with the Tasmanian sheep strain). The existence of such overlapping increases the possibility of cross-breeding in the same geographical area within the definitive host. With regard to specific genetic aspects, the two strains were independent because (1) the DTUs remained unchanged in the case of these overlapping samples, and (2) according to the nuclear markers, no heterozygote patterns were identified, indicating the absence of cross-fertilizing processes in our panel of 71 samples. However, these preliminary results should now be more profoundly confirmed by sampling a larger panel of appropriate specimens. Research on the interbreeding mode selected by both common sheep and camel strains can provide more information about their real status.

The Tasmanian sheep strain that was identified in Algeria was first characterized in Tasmania from sheep samples (Bowles et al. 1992). This genetic variant of *E. granulosus* has been considered to be geographically isolated in this island state of Australia because until now, no other variants have been described. Genotype 2 was characterized only by the use of the mitochondrial DNA markers *cox1* and *nad1*. Surprisingly, G2 was also discovered in sheep from Argentina (Rosenzvit et al. 1999; Kamenetzky et al. 2002), then in human samples from the same area (Guarnera et al. 2004), and more recently, in sheep and cattle from Romania (Bart et al. 2006). In these studies, the Tasmanian sheep strain was described as cocirculating in sympatry with the common sheep strain. In our mitochondrial dendogram, the G2 sequence showed a very low genetic divergence from the G1 sequence. The identification of G2 was only made in the present work because of the mitochondrial markers and was not connected with any particular host spectrum or geographical patterns. To date, the Tasmanian sheep strain has been described in Tasmania, Argentina, Romania, and Algeria. Thus, this variant of *E. granulosus*, much like the common sheep strain, appears to be very widespread. The two sheep strains share the same host spectrum, infecting mainly sheep, but both are also able to infect other livestock such as the camel, as described in this study.

The concept of strains is therefore controversial within the species of *E. granulosus*. In our data, the mitochondrial genetic distances observed between G1/G2 and G1/G6 were 0.7 and 11.6%, respectively, whereas an average of 13.5% was reported between G1 and *E. multilocularis*. In a study conducted by McManus (2006), the values obtained

with the same markers for G1/G4 (horse strain) and G1/G5 (cattle strain) were very similar to those observed for G1/G6 in the present work. Separating the horse and cattle strains from *E. granulosus* and assigning them their own species level (i.e., *Echinococcus equinus* and *Echinococcus ortleppi*) is accepted. The common sheep and camel strains appear to be biologically distinct and as genetically different from one another as they are from *E. multilocularis*. The taxonomic status given to all of the *E. granulosus* strains should be progressively reevaluated. It has been recently observed that the two cervid strains, G8 and G10, defined by geographical, morphological, and, more recently, genetic criteria (Lavikainen et al. 2003), seem to form, together with the pig (G7) and the camel (G6) strains, a set presumed to be a species distinct from *E. granulosus* (Thompson et al. 2005). The taxonomic name of *Echinococcus canadensis* has been proposed.

In taxonomic research, the increasing number of phylogenetic studies can be explained by their capacity to solve several of the problems previously encountered with other reference methods such as morphological, behavioral, physiological, or biochemical analyses (Kunz 2002). However, genetic discrimination is not based on the presence or absence of criteria; it involves a gradient of differences and, thus, implicitly involves the choice of a resolution level for the definition of a new relevant rank in taxonomy. In addition, the selection of the markers is important because they do not have the same discriminating characteristics (for example, mitochondrial vs nuclear). To avoid the risk of sub- or overdiscrimination, the definition of all taxonomic ranks should include geographical, epidemiological, and interbreeding patterns to reflect an epidemiological relevance. The improvement of our knowledge of taxonomic links and their evolution is not "merely an academic exercise" (McManus and Bowles 1996): it is primordial in the establishment of control or eradication campaigns against cystic echinococcosis.

Acknowledgement This work was supported by the Swiss National Science Foundation (grant no. 31-63615.00).

References

Avise JC, Walker D (1999) Species realities and numbers in sexual vertebrates: perspectives from an asexually transmitted genome. Proc Natl Acad Sci USA 96(3):992–995

Azab ME, Bishara SA, Helmy H, Oteifa NM, El-Hoseiny LM, Ramzy RM, Ahmed MA (2004) Molecular characterization of Egyptian human and animal *Echinococcus granulosus* isolates by RAPD-PCR technique. J Egypt Soc Parasitol 34(1):83–96

Bardonnet K, Piarroux R, Dia L, Schneegans F, Beurdeley A, Godot V, Vuitton DA (2002) Combined eco-epidemiological and molecular biology approaches to assess *Echinococcus granulosus* transmission to humans in Mauritania: occurrence of the 'camel' strain and human cystic echinococcosis. Trans R Soc Trop Med Hyg 96(4):383–386

Bart JM, Bardonnet K, Benchikh-Elfegoun MC, Dumon H, Dia L, Vuitton DA, Piarroux R (2004) *Echinococcus granulosus* strain typing in North Africa: comparison of eight nuclear and mitochondrial DNA fragments. Parasitology 128(Pt 2): 229–234

Bart JM, Morariu S, Knapp J, Ilie MS, Pitulescu M, Anghel A, Cosoroaba I, Piarroux R (2006) Genetic typing of *Echinococcus granulosus* in Romania. Parasitol Res 98(2):130–137

Bartholomei-Santos ML, Heinzelmann LS, Oliveira RP, Chemale G, Gutierrez AM, Kamenetzky L, Haag KL, Zaha A (2003) Isolation and characterization of microsatellites from the tapeworm *Echinococcus granulosus*. Parasitology 126 (Pt 6):599–605

Bowles J, McManus DP (1993) Molecular variation in *Echinococcus*. Acta Trop 53(3–4):291–305

Bowles J, Blair D, McManus DP (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol Biochem Parasitol 54(2):165–173

da Silva CM, Ferreira HB, Picon M, Gorfinkiel N, Ehrlich R, Zaha A (1993) Molecular cloning and characterization of actin genes from *Echinococcus granulosus*. Mol Biochem Parasitol 60(2):209–219

Dinkel A, Njoroge EM, Zimmermann A, Walz M, Zeyhle E, Elmahdi IE, Mackenstedt U, Romig T (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(5):645–653

Eckert J, Thompson RC (1997) Intraspecific variation of *Echinococcus granulosus* and related species with emphasis on their infectivity to humans. Acta Trop 64(1–2):19–34

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Guarnera EA, Parra A, Kamenetzky L, Garcia G, Gutierrez A (2004) Cystic echinococcosis in Argentina: evolution of metacestode and clinical expression in various *Echinococcus granulosus* strains. Acta Trop 92(2):153–159

Haag KL, Zaha A, Araujo AM, Gottstein B (1997) Reduced genetic variability within coding and non-coding regions of the *Echinococcus multilocularis* genome. Parasitology 115 (Pt 5):521–529

Haag KL, Araujo AM, Gottstein B, Siles-Lucas M, Thompson RC, Zaha A (1999) Breeding systems in *Echinococcus granulosus* (Cestoda; Taeniidae): selfing or outcrossing? Parasitology 118 (Pt 1):63–71

Kamenetzky L, Gutierrez AM, Canova SG, Haag KL, Guarnera EA, Parra A, Garcia GE, Rosenzvit MC (2002) Several strains of *Echinococcus granulosus* infect livestock and humans in Argentina. Infect Genet Evol 2(2):129–136

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kunz W (2002) When is a parasite species a species? Trends Parasitol 18(3):121–124

Lahmar S, Debbek H, Zhang LH, McManus DP, Souissi A, Chelly S, Torgerson PR (2004) Transmission dynamics of the *Echinococcus granulosus* sheep-dog strain (G1 genotype) in camels in Tunisia. Vet Parasitol 121(1–2):151–156

Lavikainen A, Lehtinen MJ, Meri T, Hirvela-Koski V, Meri S (2003) Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of *Echinococcus granulosus*. Parasitology 127(Pt 3):207–215

Macpherson CNL, Wachira TWM (1997) Cystic echinococcosis in Africa south of the Sahara. In: Anderson FL, Ouheli H, Kashani M (eds) Compendium on cystic echinococcosis in Africa and Middle Eastern countries with special reference to Morocco. Bringham Young University, Pravo, pp 245–277

Mayr E, Linsley EG, Usinger RL (1953) Methods and principles of systematic zoology. McGraw-Hill, New York

McManus DP (2002) The molecular epidemiology of *Echinococcus granulosus* and cystic hydatid disease. Trans R Soc Trop Med Hyg 96(Suppl 1):S151–S157

McManus DP (2006) Molecular discrimination of taeniid cestodes. Parasitol Int 55(Suppl):S31–S37

McManus DP, Bowles J (1996) Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. Int J Parasitol 26(7):687–704

M'Rad S, Filisetti D, Oudni M, Mekki M, Belguith M, Nouri A, Sayadi T, Lahmar S, Candolfi E, Azaiez R, Mezhoud H, Babba H (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(3–4):267–272

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

Obwaller A, Schneider R, Walochnik J, Gollackner B, Deutz A, Janitschke K, Aspock H, Auer H (2004) *Echinococcus granulosus* strain differentiation based on sequence heterogeneity in mitochondrial genes of cytochrome *c* oxidase-1 and NADH dehydrogenase-1. Parasitology 128(Pt 5):569–575

Rosenzvit MC, Zhang LH, Kamenetzky L, Canova SG, Guarnera EA, McManus DP (1999) Genetic variation and epidemiology of *Echinococcus granulosus* in Argentina. Parasitology 118 (Pt 5):523–530

Sadjjadi SM (2005) Present situation of echinococcosis in the Middle East and Arabic North Africa. Parasitol Int 55(Suppl):S197–S202

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425

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(4):369–381

Tassy P (1986) L'ordre et la diversite du vivant: quel statut scientifique pour les classifications biologiques? Fayard/fondation Diderot, France

Thompson RCA, McManus DP (2001). Aetiology: parasites and lifecycles (Chapter 1). WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Eckert J, Gemmel MA, Meslin FX, Pawlowski ZS and WHO. World Health Organization/World Organisation for Animal Health, Paris, France

Thompson RC, Lymbery AJ, Constantine CC (1995) Variation in *Echinococcus*: towards a taxonomic revision of the genus. Adv Parasitol 35:145–176

Thompson RC, Boxell AC, Ralston BJ, Constantine CC, Hobbs RP, Shury T, Olson ME (2005) Molecular and morphological characterization of *Echinococcus* in cervids from North America. Parasitology 132(Pt 3):439–447

Tibayrenc M (2003) Genetic subdivisions within *Trypanosoma cruzi* (discrete typing units) and their relevance for molecular epidemiology and experimental evolution. Kinetoplastid Biol Dis 2(1):12

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(4):473–479