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Cystic Echinococcosis in Algeria: Camels Act as Reservoirs of Sheep Strain *Echinococcus granulosus* Can Contribute to Human Contamination

[1]K. Kohil, [1]M.C. Benchikh ElFegoun, [2]Anne-Cécile Normand, [1]B. Babelhadj and [2]R. Piarroux

[1]Veterinary School, University of Mentouri, 25 000 Constantine, Algeria
[2]Laboratory of Parasitology, Timone Hospital, Marseille, France

Abstract: Molecular typing of strains of *Echinococcus granulosus* (*E.. granulosus)* is essential to define a strategy against cystic echinococcosis well suited for strains differ in various factors: pathogenicity for humans, PCR amplification and sequencing of mitochondrial genes of cytochrome oxidase 1 (CO1) and NADH dehydrogenase 1 (ND1) were used to characterize 42 isolates of *E.. granulosus*: 28 from animals (17 camels, four sheep, four cattle and 3 goats) collected in slaughterhouses and 14 collected on humans in surgical services in southern Algeria. The results of the study demonstrated the presence of two distinct genotypes: genotype G1 (sheep strain) (85.7%) and the G6 genotype (camel strain) (14.3%). The G1 genotype was found in 75% (3/4) of sheep, 100% (3/3) goats, 100% (4/4) of cattle and 82.3% (14/17) of camels. The G6 genotype was identified in 17.6% (3/17) camels, 25% (1/4) for sheep and 14.3% (2/14) of humans. The high frequency of G1 genotype in camels, infecting strain for humans, suggesting that camels whose prevalence and fertility of hydatid cysts is high, could represent, in the southern regions, a source of indirect transmission to humans this zoonotic strain.

Key words: *Echinococcus granulosus* · Strain Typing · Genotype · Animal Reservoirs · Algeria

INTRODUCTION

Cystic echinococcosis (CE) caused by larvae of tapeworm *E. granulosus* (Batsch, 1786), is a highly endemic zoonosis in ruminant breeding areas of North Africa. The parasite is transmitted primarily in the epidemiological cycle generally involving between dogs (definitive hosts) and livestock (sheep, cattle, goats, camels. ..) (intermediate hosts) [1]. Recent data, using molecular analysis, demonstrated a great diversity in *E. granulosus* species. Several intra-species variants identified as strains were characterized by PCR amplification and sequencing mitochondrial markers in cytochrome c oxidase 1 and NADH dehydrogenase 1 genes, from intermediate hosts of various species in various parts of the world. Each genotype, classified 1 to 10, has been associated with a particular host and epidemiological characteristics [2-6]: sheep strain (G1), cattle strain (G5), horse strain (G4), camel strain (G6)… This heterogeneity within *E. granulosus* species can influence the life cycle models, host specificity, speed of development in the definitive host, antigenicity, transmission dynamics and infectivity for humans.

The identification of strains is essential to define the suited strategy to fight against the cystic echinococcosis. In Algeria, sheep common strain and camel strain were identified [7- 9]. The involvement of cattle, besides sheep, harboring sheep strain (G1) in the human infection through the dog was demonstrated in Algeria [7, 10]. However, the importance of camels in the C.E. transmission to human through the dog is still not known. High rates of prevalence and fertility hydatid cysts: 24.8% and 100% respectively were reported in camels in southern Algeria [7]. Based on these data, it is important to define the role of camels in the transmission dynamics of *E. granulosus* to dogs (HD) and indirectly to humans.

The objective of this study is to characterize *E. granulosus* strains circulating in camels by molecular analysis in areas of southern Algeria and assess their ability in human contamination

MATERIALS AND METHODS

Hydatid cysts were collected from animals (intermediate hosts) in slaughterhouses and from humans

Corresponding Author: Benchikh El Fegoun, Laboratory of Parasitology, Timone Hospital, Marseille, France.

Fig. 1: Location of the study areas

Table1: sample Origin

	Species					
Origin	Humans	Cattle	Sheep	Goats	Camels	Total
Biskra	6		2	2		10
Ouargla	8		1	0	6	15
Tamanrasset	0	4	1	1	11	17
Total	14	4	4	3	17	42

Table 2: Characteristics of primers used

	Primer sequences	Hybridization temperatures	Target pb (1)	References
COI	5'-TTTTTTGGGCATCCTGAGGTTTAT-3' 5'-TAAAGAAAGAACATAATGAAAATG-3'	60°C	391	BOWLES *et al*., 1992
NDI	5'-AGATTCGTAAGGGGCCTAATA-3' 5'-ACCACTAACTAATTCACACTTTC-3'	45°C	471	BOWLES *et al*., 1994

in hospitals (surgical wards) located in three areas of southern Algeria (Fig. 1): Biskra, Ouargla and Tamanrasset (17 dromedaries cysts, 4 sheep cysts, 3 goats cysts, 4 cattle cysts and 14 human cysts). The origin of the analyzed samples is reported in Table 1.

Cysts Fertility Control **[**Ould Ahmed Salem *et al*., 2010**] [28]**

All cysts were fertile (p ence of protoscoleces) on microscopy magnification: 250.

DNA extraction **[** Ma *et al*., 2008**]** [29]

Genomic DNA was extracted from hydatid cysts (Protoscoleces and/ germinal membrane) obtained from fertile cysts using the High Pure PCR template Preparation (Roche Diagnostics, Mennheim, Germany), based on Proteinase K digestion. The resulting DNA was either used immediately by PCR amplification or kept at + 4 °C until use.

Molecular Analyzes

Amplification by PCR (polymerase chain reaction).

Primers Used: Two targets were amplified for each sample, with two pairs of primers NDI (1 gene region of the mitochondrial NADH dehydrogenase) and COI (portion of the mitochondrial gene encoding cytochrome c oxidase subunit 1). The characteristics of these primers are indicated in Table 2.

The amplification conditions were as following: an initial denaturation step (30 s at 94°C) followed by 40 successive cycles of denaturation (30 s at 95°C), annealing (30 s at the annealing temperature) and elongation (30-75 sec at 72°C) and a final elongation of 5 min at 72°C [29]. The size and the specificity of the amplified products were evaluated by electrophoresis in 1.5% (w / v) agarose frozen Tris-acetate / EDTA. 2,5µl of each amplified fragment was then purified for 15 minutes at 37°C and 15 minutes at 80°C. The purified DNA was

then sequenced using cycle sequencing kit includes: DYEnamic-ET terminator (Amersham Pharmacia Biotech Europe GmbH, Freiberg, Germany). The detection of fragments was performed in an automatic sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter, Fullerton, CA, USA) [29]. Electropherograms obtained from one primer were studied in parallel with those obtained with the primer complementary. The complete sequences were then compared with each other and with the previously published sequences in the Genbank database (http://www.ncbi.nlm.nih.gov) with the BLAST system.

RESULTS AND DISCUSSION

PCR: The two PCR fragments of genomic DNA mitochondrial (COI and NDI) were amplified for all samples. A single DNA band of the expected molecular weight was obtained by PCR with each of the two pairs of primers and sequencing the amplified target was possible for all samples analyzed.

Based on the genomic sequences Cox1 and nad1 two genotypes were identified: - The first genotype was obtained from 36 samples (14/17 camels, sheep 3/4, 4/4, cattle, goats and 3/3 12/14 humans) of 42 in total, or 85.7% (Table 3). The sequence obtained from these 36 samples showed 99.2% to 100% homology (2-0 difference / 391 base pairs) between them, so that 99% to 100% homology found with the sequence COI partial genomic genotype (G1), sheep strain of *E. granulosus* identified from other Algerian samples [7, 9].

The second genotype (G6) was obtained from 6 samples (3/17 camels, sheep and 1/4 2/14 humans) or 14.3% (Table 3). One hundred percent of homology found between this sequence and the sequences corresponding to the genotype camel strain (G6) identified in camels in Algeria [7] in Mauritania [11] and other African countries [4].

Sequencing of two targets amplified with two pairs of primers Cox1 and Nad1 confirmed with 42 new samples the existence of two *E.granulosus* strains in southern Algeria:

- Sheep strain (G1) circulating in sheep, cattle, goats and in more than half of the camels, transmissible to humans.

The ubiquity of this strain was reported in several countries worldwide: in North Africa: in Tunisia [12, 13], in Algeria [7, 9, 10, 14] and in Libya [15]. In Iran, this ubiquitous character of the genotype (G1) of *E. granulosus* has been commonly affecting humans, sheep, cattle, goats and sometimes camels [12, 16, 18]. In the south east of Iran, the predominance of the genotype G1 was recorded after amplification and sequencing of

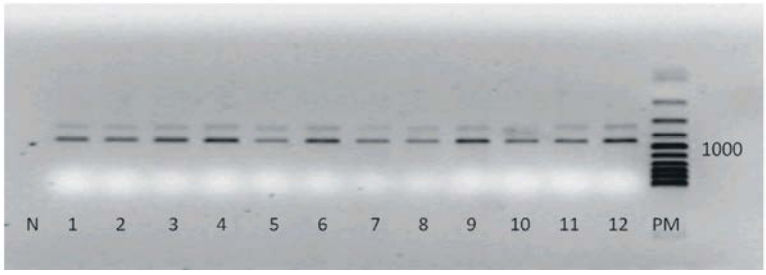

Fig. 2: Electrophoresis of PCR products using primers CO1 / CO2, PM: molecular weight marker (in base pairs), 1 to 3: camel samples; 4, 5: sheep samples; 6: goat sample; 7 to 9: bovine samples; 10 to 12: human samples.

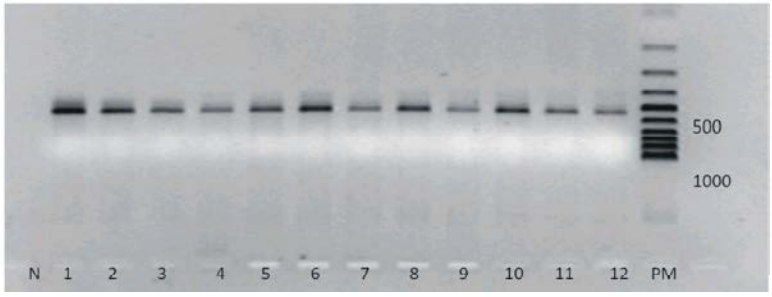

Fig. 3: Electrophoresis of PCR products using primers ND1 / ND2, PM: molecular weight marker (in base pairs), 1 to 3: camel samples; 4, 5: sheep samples; 6: goat sample; 7 to 9: bovine samples; 10 to 12: human samples.

Table 3: Features and genotyping results of samples

Hôtes	Origine	Amorces	n°accession1	génotype	%	n°accession2	génotype	%	n°accession3	Génotype	%	%
1	Camelins	Ouargla	nad									100
			cox	AB688142.1	G6	99	AB208063.1	G6	99	AB274020.1	G6 100	100
2	Camelins	Ouargla	nad									100
			cox	AB688142.1	G6	99	AB208063.1	G6	99	AB274020.1	G6 100	100
3	Camelins	Ouargla	nad	HM055626.1	G1	100	AF297617.1	G1	100	KC579441.1	G1 100	100
			cox	AB786664.1	G1	99	AB688617.1	G1	99	AB688616.1	G1 100	100
4	Camelins	Ouargla	nad	HM055626.1	G1	100	AF297617.1	G1	100	KC579441.1	G1 100	100
			cox									100
5	Camelins	Ouargla	nad	EF367324.1	G1	100	EF367323.1	G1	100	EF367322.1	G1 100	100
			cox	EF367262.1	G1	99		G1	99	KC109659.1	G1 100	100
6	Camelins	Ouargla	nad									100
			cox				EF367259.1					100
7	Camelins	Tamanrasset	nad						99	JQ317990.1	G7 100	100
			cox	DQ356884.1	G6	99	AB688142.1	G6	99	JQ317990.1	G7 100	100
8	Camelins	Tamanrasset	nad	EF367324.1	G1	99	EF367323.1	G1	99	EF367322.1	G1 100	100
			cox	AB688600.1	G1	99	AB688599.1	G1	99	AB688595.1	G1 100	100
9	Camelins	Tamanrasset	nad	EF367324.1	G1	98	EF367323.1	G1	98	EF367322.1	G1 100	100
			cox	EF367259.1	G1	99	KC109659.1	G1	99	AB688599.1	G1 100	100
10	Camelins	Tamanrasset	nad	HM055626.1	G1	99	AF297617.1	G1	99	JF946624.1	G1 100	100
			cox	KC109655.1	G1	98	KC109654.1	G1	98	KC109653.1	G1 100	100
11	Camelins	Tamanrasset	nad	HM055626.1	G1	98	AF297617.1	G1	99	EF367301.1	G1 100	100
			cox	AY386210.1	G1	97	AB688621.1	G1	97	AB688620.1	G1 100	100
12	Camelins	Tamanrasset	nad	JF946624.1	G1	99	JF946623.1	G1	99	JF946622.1	G1 100	100
			cox	AB491423.1	G1	98	DG356879.1	G1	98	AB688921.1	G1 100	100
13	Camelins	Tamanrasset	nad	JF946624.1	G1	98	JF946623.1	G1	98	JF946622.1	G1 100	100
			cox	AB491423.1	G1	99	EF367292.1	G1	99	DQ356879.1	G1 100	100
14	Camelins	Tamanrasset	nad	EF367324.1	G1	100	EF367323.1	G1	100	EF367322.1	G1 100	100
			cox	EF367262.1	G1	99	EF367259.1	G1	99	KC109659.1	G1 100	100
15	Camelins	Tamanrasset	nad									100
			cox	AB688621.1	G1	99	AB688617.1	G1	99	AB688616.1	G1 100	100
16	Camelins	Tamanrasset	nad	EF367312.1	G1	100	EF367311.1	G1	100	JF946624.1	G1 100	100
			cox									100
17	Camelins	Tamanrasset	nad	JF946624.1	G1	99	JF946623.1	G1	99	JF946622.1	G1 100	100
			cox									100
18	Humains	Biskra	nad	HM749617.1	G6	91	HM749616.1	G6	91	HM749615.1	G6 100	100
			cox	AB688142.1	G6	95	JQ317990.1	G6	95	JQ356717.1	G6 100	100
19	Humains	Biskra	nad	HM749617.1	G6	89	HM749616.1	G6	89	HM749615.1	G6 100	100
			cox	AB688142.1	G6	99	AB208063.1	G6	99	AB274020.1	G6 100	100
20	Humains	Biskra	nad									100
			cox	AB688621.1	G1	99	AB688617.1	G1	99	AB688616.1	G1 100	100
21	Humains	Biskra	nad									100
			cox	AB688617.1	G1	99	AB688616.1	G1	99	AB688614.1	G1 100	100
22	Humains	Biskra	nad									100
			cox	AB688617.1	G1	99	AB688616.1	G1	99	AB688614.1	G1 100	100
23	Humains	Biskra	nad	JF946624.1	G1	99	JF946623.1	G1	99	JF946622.1	G1 100	100
			cox									100
24	Humains	Ouargla	nad	GQ358013.1	G1	100	GQ358012.1	G1	100	GQ358011.1	G1 100	100
			cox									100
25	Humains	Ouargla	nad	HM055626.1	G1	100	AF297617.1	G1	100	JF946624.1	G1 100	100
			cox	AB688618.1	G1	100	JF906165.1	G1	100	AB491456.1	G1	100
26	Humains	Ouargla	nad	GQ358013.1	G1	100	GQ358012.1	G1	100	GQ358011.1	G1	100
			cox									100
27	Humains	Ouargla	nad	EF367312.1	G1	100	EF367311.1	G1	100	HM055626.1	G1	100
			cox	AB688621.1	G1	99	AB688620.1	G1	99	AB688617.1	G1	100
28	Humains	Ouargla	nad	HM055626.1	G1	100	AF297617.1	G1	100	JF946624.1	G1	100
			cox	AB688618.1	G1	99	DQ356881.1	G1	99	DQ356879.1	G1	100

Table 3: Continued

Hôtes	Origine	Amorces	n°accession1	génotype	%	n°accession2	génotype	%	n°accession3	Génotype	%	%
29	Humains	Ouargla	nad	EF367301.1	G1	99	JF946624.1	G1	100	JF946623.1	G1	100
			cox	AB688618.1	G1	99	JF906165.1	G1	100	AB491456.1	G1	100
30	Humains	Ouargla	nad	HM055626.1	G1	100	JF946624.1	G1	100	JF946624.1	G1	100
			cox									100
31	Humains	Ouargla	nad	JF946624.1	G1	99	JF946623.1	G1	99	JF946622.1	G1	100
			cox	AB688617.1	G1	99	AB688616.1	G1	99	AB688614.1	G1	100
32	Bovins	Tamanrasset	nad	HM055626.1	G1	99	AF297617.1	G1	99	JF946624.1	G1	100
			cox	AB688621.1	G1	99	AB688617.1	G1	99	AB688616.1	G1	100
33	Bovins	Tamanrasset	nad	EF367324.1	G1	96	EF367323.1	G1	96	EF367322.1	G1	100
			cox	AB688617.1	G1	99	AB688616.1	G1	99	AB688614.1	G1	100
34	Bovins	Tamanrasset	nad	JF946624.1	G1	99	JF946623.1	G1	99	JF946622.1	G1	100
			cox									100
35	Bovins	Tamanrasset	nad	HM055626.1	G1	100	AF297617.1	G1	100	JF946624.1	G1	100
			cox									100
36	Ovins	Biskra	nad	HM055626.1	G1	99	AF297617.1	G1	99	KC579441.1	G1	100
			cox									100
37	Ovins	Ouargla	nad	EF367312.1	G1	100	EF367311.1	G1	100	JF946624.1	G1	100
			cox	AB491423.1	G1	99	DQ356879.1	G1	99	AB491421.1	G1	100
38	Ovins	Tamanrasset	nad									100
			cox	AB688142.1	G6	99	AB208063.1	G6	99	AB274020.1	G6	100
39	Ovins	Biskra	nad	HM055626.1	G1	99	AF297617.1	G1	99	JF946624.1	G1	100
			cox									100
40	Caprins	Biskra	nad	HM055626.1	G1	99	AF297617.1	G1	99	JF946624.1	G1	100
			cox	AB688617.1	G1	100	AB688616.1	G1	100	AB688614.1	G1	100
41	Caprins	Biskra	nad	HM055626.1	G1	99	AF297617.1	G1	99	JF946624.1	G1	100
			cox	AB688617.1	G1	99	AB688616.1	G1	99	AB688614.1	G1	100
42	Caprins	Tamanrasset	nad									100
			cox	AB688621.1	G1	100	AB688617.1	G1	100	AB688616.1	G1	100

mitochondrial genes cox1and nad1 of samples of ruminants: sheep (86.7%), cattle (80%), camels (44.4%) and goats (100%) [12]. Similarly in Turkey, the genotype G1 is common in humans and sheep (17/22 analyzed), the samples were examined by the sequencing of DNA of four mitochondrial genes [19]. The genotype G1 is responsible of the vast majority of the human cystic echinococcosis worldwide (88.44%), where its spreading is cosmopolite nd it is usually related to human transmission via sheep, like intermediate hosts [20]. However, the results of this present study showed the participation of camels alongside other domestic ruminants (sheep and cattle) in maintaining the life cycle of the common sheep strain in the southern regions of Algeria and their involvement in the human contamination. This corroborates the reported data in molecular studies done in Iran where camels are susceptible to host the common sheep strain. In fact, the typage of the strains of *E.granulosus* indicates occasionally high frequencies of the genotype G1 in this species: 44.4% [17] and 66.7% [21] of the camels were found infected by the genotype G1suggesting the participation of camels in the dynamic of the transmission of this zoonotic strain to humans via the dog.

In the present study; the camel strain (G6) was identified in camels and was circulating occasionally in the other ruminants (1sheep sample in the present study). This strain was isolated in both human sample and was similar to these found in camels in Touggourt region in a preliminary study [7] and in camels, cattle and humans in Mauritania [11]. The genotype G6 is generally well suited to camels but can also circulate in other ruminants (cattle, sheep and goats) and infect humans [11, 22-24]. The predominance of this camel strain was reported in animals and humans in some countries: the genotype G6 was detected in 100% of isolates of animal origin and in 96.8% human isolates in Egypt [24]. This strain was detected in 98.7% and 100% of hydatid cysts collected in livestock and humans Sudan respectively [23]. Ahmed *et al.* [25] confirmed in a new phylogenetic analysis, the predominance of the genotype G6 where 98% of the samples of the cysts of camels correspond to this camel strain (G6). Even though the camels constitute potential reservoir of the camel strain (G6), other species especially goats could represent an important source. This strain is well known in Latin America (Argentina), where it seems that it is mainly hosted by goats [26].

In Algeria, its occurrence (G6) results in involvement in the programs of local control due to the duration of the prepatent period, shorter in dogs in comparison to that of the common sheep strain (G1).

CONCLUSION

This new study demonstrated the participation of camels alongside other domestic ruminants in the maintaining of life cycle of the common sheep strain and their involvement in human contamination. The presence of genotype G1 in camels, an infective strain for humans, suggests that camels whose prevalence and fertility of cysts is usually high, could represent, in the southern regions, a source of indirect transmission to humans of this zoonotic strain. Control programs should take into consideration the potential risk of this species in maintaining the life cycle of the parasite particularly in the human contamination. Moreover, the pace of medical treatment of dogs (HD) should be adapted to the length of the speed of development of the adult tapeworm of camel strain (G6) which is slightly shorter (40 days instead of 45 days for genotype G1).

A better knowledge of the evolution of sheep and camel strains requires typing new camels and human samples in southern Algeria. This will allow us to redefine the cycle of *E. granulosus* and propose appropriate and effective prophylactic measures.

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