Molecular and Cellular Probes 25 (2011) 49–54

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr

A first insight into the genotypes of *Echinococcus granulosus* from humans in Mongolia

Abdul Jabbar [a,*], Myadagsuren Narankhajid [b], Matthew J. Nolan [a], Aaron R. Jex [a], Bronwyn E. Campbell [a], Robin B. Gasser [a,*]

[a] Department of Veterinary Science, The University of Melbourne, Victoria 3030, Australia
[b] Department of Medical Biology, School of Biomedicine, University of Health Sciences, Ulaanbaatar, Mongolia

ARTICLE INFO

Article history:
Received 8 October 2010
Received in revised form
3 November 2010
Accepted 3 November 2010
Available online 12 November 2010

Keywords:
Echinococcus granulosus
Cystic echinococcosis (CE)
Mitochondrial DNA
Population genetics
Epidemiology
Mongolia

ABSTRACT

Polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) and targeted sequencing were employed to genetically classify *Echinococcus granulosus* cysts from humans from 12 provinces in Mongolia using two DNA loci, designated p*cox*-1 and p*nad*-1, within the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*-1) and NADH dehydrogenase subunit 1 (*nad*-1) genes, respectively. SSCP analysis of p*cox*-1 and p*nad*-1 amplicons produced from genomic DNA samples from individual *E. granulosus* cysts ($n = 50$) from individual humans displayed four distinct electrophoretic profiles for each p*cox*-1 and p*nad*-1. The direct sequencing of selected amplicons representing each of these profiles defined four distinct sequence types for each locus, present in four different combinations (designated as haplotypes M1–M4) for all 50 cyst isolates. Phylogenetic analysis of concatenated sequence data for these four haplotypes, including well-defined reference sequences, inferred that 68% of the cyst isolates belonged to the G1–G3 complex of *E. granulosus* (or *E. granulosus sensu stricto*), whereas the remaining (32%) were linked to the G6–G10 complex (or *Echinococcus canadensis*). Humans infected with *E. granulosus* cysts of the G1–G3 complex originated mainly from the eastern regions of Mongolia, whereas those harbouring cysts of the G6–G10 complex were from the western part of this country. The present study provides a first glimpse of the genetic composition of *E. granulosus* from humans in Mongolia, and forms a foundation for future studies of the epidemiology and ecology of the parasite(s) in animals and humans in this and surrounding countries.

1. Introduction

Cystic echinococcosis (CE) of humans and other mammals is a socioeconomically important disease caused by the infection with the larval (cyst) stage of the canid tapeworm *Echinococcus granulosus* (Eucestoda: Taeniidae) [1–3]. Humans and intermediate hosts, such as sheep, become infected by ingesting the parasite eggs originating from the faeces from a canid (definitive) host. In the intermediate host, the eggs hatch to release oncospheres (=hexacanth embryos), which migrate through the intestinal wall and into vessels to then be disseminated passively via the blood stream to various organs, particularly lung and liver, in which they develop and grow to become fluid-filled cysts (often ~3–10 cm in diameter). In humans, CE is often characterised by an asymptomatic phase (months to years), followed by acute clinical signs, such as pain or swelling, as a consequence of the pressure that growing cysts exert on surrounding parenchyma or tissues [1,3]. The clinical manifestation depends on the organ(s) affected, the localisation, and the number and size of the cysts present. CE can be life-threatening, particularly if cysts rupture and their contents (fluid, protoscoleces and/or brood capsules) spill into the peritoneal cavity, resulting in an anaphylactic shock [3] and/or the establishment of secondary CE [1,3]. Globally, this disease affects ~2–3 million people and also causes major economic losses to the meat and livestock industries due to the condemnation of infected livers and lungs from livestock animals at slaughter [3–5].

The genetic characterisation of *E. granulosus* populations is central to an understanding of the transmission patterns of the parasite(s) between dogs/other canid definitive hosts and humans/intermediate mammalian hosts, and assists significantly in the diagnosis and control of CE [6,7]. Various DNA-based tools, particularly polymerase chain reaction (PCR)-coupled mutation scanning and/or sequencing of nuclear and mitochondrial gene regions, have

* Corresponding authors. Tel.: +61 3 97312022; fax: +61 3 97312366.
E-mail addresses: jabbara@unimelb.edu.au (A. Jabbar), robinbg@unimelb.edu.au (R.B. Gasser).

0890-8508/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.mcp.2010.11.001

been applied to the genetic classification of *Echinococcus* species and genotypes [8–12]. To date, 10 distinct genotypes (designated G1–G10) of *E. granulosus* (*sensu lato*) have been defined based on mitochondrial DNA studies in a range of countries/continents, including parts of Africa, Asia, Australia, Europe, South America, and the USA [11–19]. However, because no molecular data are available on *E. granulosus* in many countries of Africa, South America and Asia (such as the Middle East, central, southern and south-eastern Asia), the full extent of genetic variation within *E. granulosus* might not be known, and new genotypes could be discovered. For instance, although CE is recognised to be endemic in some parts of Mongolia, with prevalences in humans ranging from 0.2 to 18% [20–23], nothing is known about the genetic composition of *E. granulosus* populations in this country. In the present study, we conducted the first molecular analysis of cysts from humans from 12 different provinces in Mongolia and studied the relationships of the genotypes defined with currently recognised genotypes/species by phylogenetic inference.

2. Methods

Mongolia covers an area of ~1.6 million km^2 and is divided into 21 provinces and 334 counties. Almost 90% of Mongolia is pasture, desert and/or mountains, and l0% of the land is covered by forests; only ~l% of the land is cultivable. Animal husbandry is the backbone of Mongolia's economy and most households in rural regions maintain livestock (including sheep, goats, horses, cattle and camels) for their livelihood.

Cysts, consistent morphologically with those of *E. granulosus*, were collected from humans from 12 different provinces in Mongolia (Fig. 1). Ethics approval was granted (02.07.2009) by the University of Health Sciences, Ulaanbaatar, Mongolia. Fifty individual cysts were collected from the livers or lungs from individual human patients during surgery in hospitals. Protoscoleces, brood capsules or germinal layers from individual cysts were washed extensively in physiological saline, fixed in 50% ethanol and then stored at −20 °C. Prior to DNA extraction, the ethanol was removed, and a packed volume (50–150 μl) of protoscoleces or germinal layer from individual cysts was washed three times (45 min) in H_20, and then suspended in ~200 μl of 20 mM Tris–HCl (pH 8.0), 100 mM EDTA, 1% sodium dodecyl-sulphate (SDS) containing 10 mg/ml proteinase K (Amresco Inc., USA) and incubated at 40 °C for ~18 h. Total genomic DNA was isolated from the homogenised suspension using a mini-column (Wizard® DNA Clean-Up System, Promega, USA), according to the manufacturer's protocol.

Two mitochondrial loci were amplified from genomic DNA by PCR. The first locus (designated p*cox*-1; ~450 bp) within the cytochrome *c* oxidase subunit 1 (*cox*-1) gene was amplified using primers JB3 and JB4.5 [15]; the second locus (designated p*nad*-1; ~400 bp) within the NADH dehydrogenase subunit 1 (*nad*-1) gene was amplified utilising primers MS1 and MS2 [11]. PCR was conducted in a 50 μl volume containing 10 mM Tris–HCl (pH 8.4), 50 mM KCl (Promega), 3.5 mM of $MgCl_2$, 200 μM of each deoxynucleotide triphosphate (dNTP), 50 pmol of each primer and 1 U of G*oTaq* polymerase (Promega) under the following cycling conditions: 94 °C/30 s (denaturation); 52 °C (p*cox*-1) or 50 °C (p*nad*-1)/30 s (annealing); 72 °C/30 s (extension) for 30 cycles, followed by a final extension at 72 °C/10 min. For each set of PCRs, negative (no-DNA) and known positive controls were included. No amplification was detected in any of the negative control reactions at any stage of this study.

The p*cox*-1 and p*nad*-1 amplicons produced (separately) from individual genomic DNAs from individual cysts were subjected to single-strand conformation polymorphism (SSCP) analysis (protocol B) [24] to directly display sequence variation among amplicons. The mutation detection rate of this method has been inferred to be 100% for both pcox-1 and p*nad*-1 of *E. granulosus* [11,12]. For each locus, 2–5 amplicons representing each unique SSCP profile were selected, treated with shrimp alkaline phosphatase and exonuclease I (Fermentas Inc., USA) [25] and then subjected to bi-directional, automated sequencing (BigDye® Terminator v.3.1 chemistry, Applied Biosystems, Foster City, California, USA) using (separately) the same primers as employed in PCR. The quality of each sequence was assessed by appraising its corresponding electropherogram using the program BioEdit [26].

Previous analyses [11,12] revealed a concordance in genotypic classification between p*cox*-1 and p*nad*-1 as well as the applicability of combined sequence data to estimate haplotypic variability within *E. granulosus (sensu lato)*. Thus, individual pairs of p*cox*-1 and p*nad*-1 sequence types representing all cysts studied here allowed

Fig. 1. A map of Mongolia showing the geographical origins of *Echinococcus granulosus* isolates ($n = 50$) from humans used in the present study. Different shape(s) for each locality represent(s) a unique profile based on single-strand conformation polymorphism (SSCP) and targeted sequencing of p*cox*-1 and p*nad*-1; the number inside each shape indicates the number of samples from each location. The sequence linked to each unique SSCP profile is represented by its GenBank accession number for p*cox*1 (HQ231396–HQ231399) and p*nad*-1 (HQ231400–HQ231403).