DOI : 10.5958/2277-8934.2018.00032.2 *Vol 25 No 2, p 245-248*

FIRST REPORT ON INCIDENCE OF *Echinococcus canadensis* G6 STRAIN FROM A DROMEDARY CAMEL OF INDIA

Shirish D. Narnaware[1] and Shyam S. Dahiya[2]

[1]ICAR- National Research Centre on Camel, Post Bag No. 07, Jorbeer, Bikaner 334001, Rajasthan, India
[2]ICAR- Directorate on Foot and Mouth Disease, IVRI Campus, Mukteswar, District Nainital Uttarakhand, India 263138

ABSTRACT

In the present study, a hydatid cyst was reported in the lung of an Indian dromedary camel during postmortem examination and it was investigated for genotype analysis. Total DNA was extracted from protoscolices and associated germinal layers of hydatid cyst and used in PCR amplification of mitochondrial *cytochrome C oxidase subunit 1* (*cox1*) gene. The PCR product was purified, sequenced and analysed using bioinformatics tools. The phylogenetic analysis showed that the isolate clustered with *Echinococcus canadensis* genotype 6 (G6). The circulation of the camel genotype (G6) in the Indian one-humped camel is reported for the first time in this study, which indicates that dromedary camel has an important role in the continuation of the *E. canadensis* life cycle.

Key words: Camel, cox 1 gene, *Echinococcus canadensis*, G6 strain, genotype, India

Hydatidosis is a zoonotic disease caused by larval stages of cestodes belonging to the genus *Echinococcus*. It is characterised by long term growth of the metacestode (hydatid cysts) in the intermediate host (McManus *et al,* 2003). It is a wide spread infection throughout the world including India and is found to occur in all domestic livestock including camels and cattle (Ibrahem *et al,* 2002; Sharma *et al,* 2013). Thus camels infected with cystic echinococcosis (CE) may represent an important source of transmission to dogs and hence indirectly to man (Lahmar *et al,* 2004). Because of the involvement of the vital organs, CE in humans is considered as a critical public health problem. Moreover, CE represents one of the neglected tropical diseases (Wahlers *et al,* 2012). Although cystic echinococcosis is highly endemic in human and animal population in India, there is still scarce information about species and/ or genotypes of the *Echinococcus granulosus* complex that infect humans and animals in India (Sharma *et al,* 2013).

Currently, ten distinct genotypes of *Echinococcus granulosus* (EG) designated as G1-G10 have been described worldwide on the basis of genetic diversity related to nucleotide sequences of the mitochondrial *cytochrome C oxidase subunit 1* (*cox1*) and NADH dehydrogenase subunit 1 (NADH 1) genes (Ahmed *et al,* 2013). These different genotypes are associated with distinct intermediate hosts including sheep, pigs, cattle, horses, camels, goats and cervids. Different genotypes would probably exhibit different antigenicity, transmission profiles and sensitivity to chemotherapeutic agents as well as different pathological consequences (Thompson and McManus, 2002). Therefore, due to epidemiological implementation and control strategies, it is essential that circulating EG genotypes in a given area of endemicity should be clearly defined (McManus *et al,* 2003). In present study a genotypic analysis of a hydatid cyst recovered from lung of a dromedary camel is reported.

Materials and Methods

An adult female dromedary camel from an organised herd located at Bikaner district of Rajasthan, India was presented for routine post mortem examination. This camel was raised under semi-intensive system of management and was regularly sent for grazing in nearby field area inhabited by large number of stray dog population. On postmortem, the camel was found to have single hydatid cyst in lung which was collected intact for determination of cyst fertility, histopathology and genotype identification. For determination of cyst fertility, the surface of the cyst was sterilised with alcoholic iodine solution and the cyst wall was penetrated using a large size needle and the contents

SEND REPRINT REQUEST TO SHIRISH D. NARNAWARE email: sdnarnaware1@gmail.com

were transferred into a sterile 15 ml centrifuge tube using a syringe. To ensure the maximum collection of protoscolices, the cyst was flushed 3-4 times with sterile normal saline. The contents were centrifuged at 5000 rpm for 5 min. The supernatant was discarded and a drop of sediment was placed on slide and examined under microscope. The part of the cyst along with lung tissue was also collected in 10% formal saline for histopathology. The formalin fixed tissue samples were embedded in paraffin, cut into 4-5 micron sections and stained with hematoxylin and eosin stain.

For genotype analysis, genomic DNA was extracted from protoscolices and associated germinal layers of hydatid cysts using a commercial kit (PureLink™ genomic DNA Kit, Thermo Fisher Scientific). The DNA was subjected to PCR for amplification of *cox1* gene of *Echinococcus* by using the specific primer sequence and the reaction procedure described previously (Pednekar *et al,* 2009). The amplified PCR product was purified and sequenced. The sequence was submitted in genebank database (accession number KY436827) and was aligned with available GenBank sequences for the *E. granulosus* genotypes. The phylogenetic analysis of the nucleotide sequence of the cox1 gene of *Echinococcus* isolated from Bikaner, Rajasthan was done by the maximum likelihood statistical method using molecular evolutionary genetics analysis software (MEGA 6) (Tamura *et al,* 2013).

Results

Grossly, the cyst collected from lung was thick walled, unilocular, tennis ball sized, spherical in shape, off white in colour with presence of slightly turbid fluid. The protoscolices collected from cystic fluid contained four suckers and a rostellum that has about 25–50 hooks. The histopathology of cyst revealed that the cystic wall consisted of three layers which are innermost germinal layer, eosinophilic laminated membrane beneath the germinal layer and outer fibrous layer with dense fibrovascular tissue, respectively. The histopathological changes in lungs were thickening of alveolar and bronchiolar wall and fibrous tissue proliferation around cystic wall.

The PCR for cox1 gene yielded amplification product of 434 bp. Molecular characterisation revealed that the strain involved in the infection is most identical to the G6 camel strain. In fact, the analysis of the variable sites of the cox1 sequences obtained for the sample indicates 99.5% nucleotide identity to G6 strain. The sequence obtained from the PCR products was found to align with corresponding regions for cox1 genes in the GenBank confirming

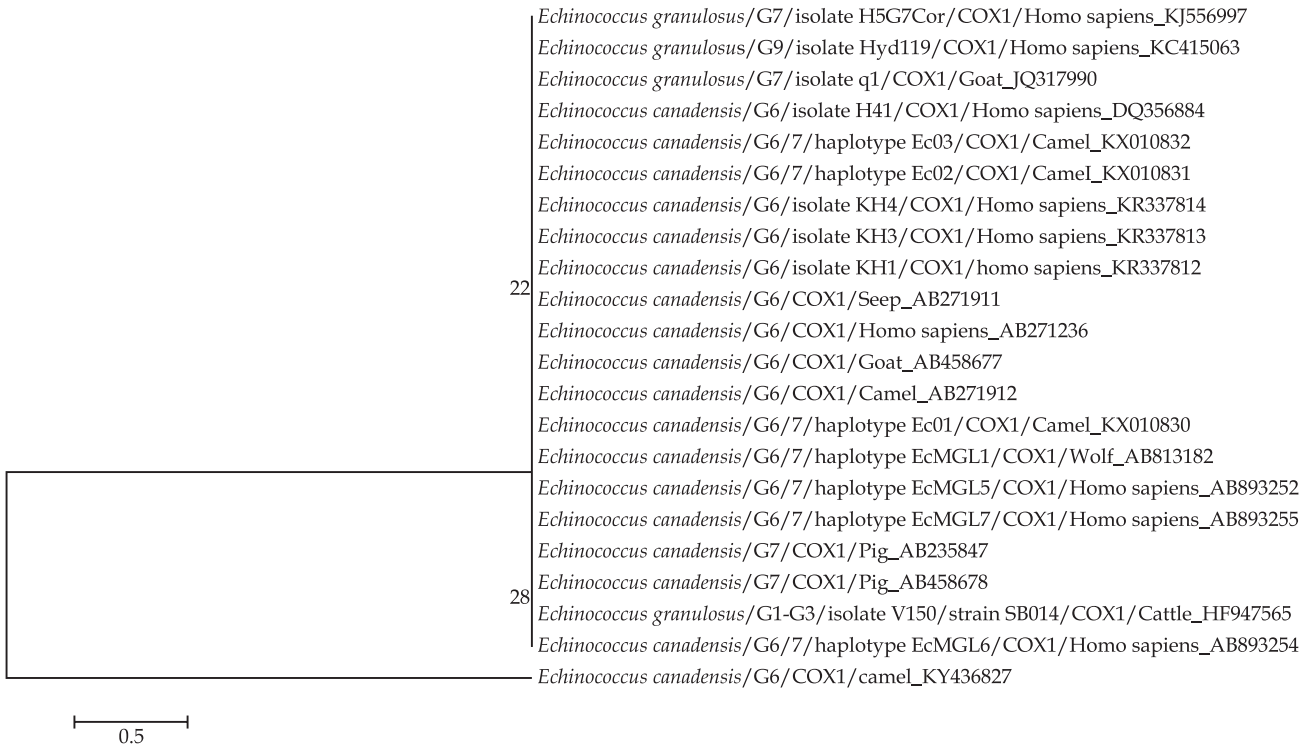


Fig 1. Phylogenetic analysis cox 1 gene of *E. canadensis* isolate from Bikaner. A total of 22 sequences were taken from GenBank. The phylogenetic tree was constructed by maximum likelihood method using Tamura-Nei model in Mega 6 program including 500 replica of bootstrap.

the cysts to contain the EG complex. Aligned with BioEdit, partial sequences for cox1 showed 100% homology in this study. To investigate for the relationship between this EG isolate and the other EG genotypes identified globally, phylogenetic tree was constructed which showed that the EG isolate (NRCC_EC_ KY436827) clustered with *E. canadensis* genotype G6 (Fig. 1).

Discussion

In Rajasthan, camels are owned by pastoralists as a source of milk, carrying loads and riding animals. Very scarce research has been conducted to evaluate the role played by camels in transmission of parasitic infections with special emphasis on cystic echinococcosis in India. The incidence of camel strain in Indian dromedary camel suggest that camel seem to play an important role in the transmission cycle of the parasite and the epidemiology of the disease particularly in camel rearing areas of Rajasthan. In the neighbouring country of Pakistan the prevalence and fertility of hydatid cysts was found highest in camels compared to other livestock species (Latif *et al,* 2010). However, phylogenetic analysis of the cox1 gene revealed that the common sheep strain (G1) and buffalo strain (G3) are cycling among camels of Pakistan and these strains are highly adapted to goats, camels and cattle (Latif *et al,* 2010).

In the present study, the phylogenetic analysis revealed the first report of *Echinococcus canadensis* camel genotype (G6) in the camels of India. This indicated that G6 genotype should equally be considered as an infectious form of EG-complex in the one humped camels in India. This finding provides an alarming evidence for the circulation of the camel genotype in the one humped camels. The close relationship between stray dogs and camels in the grazing area seem to play an important role in transmission and continuation of life cycle of *E. canadensis* in the present study.

Of the ten genotypes of EG, the strains to date reported from livestock population of India are the sheep (G1) (widely distributed), pig (G2), buffalo (G3) (widely distributed) and cattle (G5) strains (Bhattacharya *et al,* 2007; Gudewar *et al,* 2009; Pednekar *et al,* 2009; Singh *et al,* 2012). Whereas, sheep (G1), buffalo (G3), cattle (G5) and camel (G6) strains were reported from human population in India (Sharma *et al,* 2013). There is only a single case of incidence of camel strain from a human case from Rajasthan region of India (Sharma *et al,* 2013) which indicates potential of this strain to infect human population of camel rearing regions. In order to understand the transmission cycle, zoonotic potential of camel strain and epidemiology of the disease in India, a vigilant approach is required involving large number of human and camel population with full genome sequencing of some of the representative samples. This will be helpful to design and implement the control strategy for the disease.

Conclusion

The G6 genotype confirms a geographical distribution in India and demonstrates that camel can act as intermediate hosts. Therefore the importance of genotyping the isolates of *E. granulosus* complex has to be stressed in order to assess the contribution of G6 strain to the epidemiology of human hydatidosis.

Acknowledgements

The authors are grateful to Director, ICAR National Research Centre on Camel, Bikaner, India for providing necessary facilities to carry out the research work.

References

Ahmed ME, Eltom KH, Musa NO, Ali IA, Elamin FM, Grobusch MP and Aradaib IE (2013). First report on circulation of *Echinococcus ortleppi* in the one humped camel (*Camelus dromedarius*), Sudan. BMC Veterinary Reseasrch 9:127.

Bhattacharya D, Bera AK, Bera BC, Maity A and Das SK (2007). Genotypic characterisation of Indian cattle, buffalo and sheep isolates of *Echinococcus granulosus*. Veterinary Parasitology 143:371-374.

Gudewar J, Pan D, Bera AK, Das SK, Konar A, Rao JR, Tiwari AK and Bhattacharya D (2009). Molecular characterisation of *Echinococcus granulosus* of Indian animal isolates on the basis of nuclear and mitochondrial genotype. Molecular Biology Reports 36:1381-1385.

Ibrahem M, Rafiei A, Dar F, Azwai S, Carter S and Craig P (2002). Serodiagnosis of cystic echinococcosis in naturally infected camels. Parasitology 125:245-251.

Lahmar S, Debbek H, Zhang LH, Mcmanus DP, Souissi A, Chelly S and Torgerson PR (2004). Transmission dynamics of the *Echinococcus granulosus* sheep-dog strain (G1 genotype) in camels in Tunisia. Veterinary Parasitology 121:151-156.

Latif AA, Tanveer A, Maqbool A, Siddiqi N, Kyaw-Tanner M and Traub RJ (2010). Morphological and molecular characterisation of *Echinococcus granulosus* in livestock and humans in Punjab, Pakistan. Veterinary Parasitology 170:44-49.

McManus DP, Zhang W, Li J and Bartley PB (2003). Echinococcosis. Lancet 362:1295-1304.

Pednekar RP, Gatne ML, Thompson RC and Traub RJ (2009). Molecular and morphological characterisation of

Echinococcus from food producing animals in India. Veterinary Parasitology 165:58-65.

Sharma M, Sehgal R, Fomda BA, Malhotra A and Malla N (2013). Molecular characterisation of *Echinococcus granulosus* cysts in north Indian patients: Identification of G1, G3, G5 and G6 genotypes. PLoS Neglected Tropical Diseases 7:e2262.

Singh BB, Sharma JK, Ghatak S, Sharma R, Bal MS, Tuli A and Gill JPS (2012). Molecular epidemiology of Echinococcosis from food producing animals in north India. Veterinary Parasitology 186:503-506.

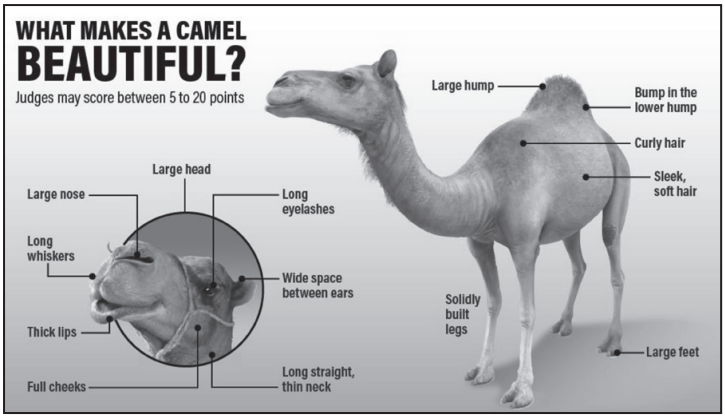
Tamura K, Stecher G, Peterson D, Filipski A and Kumar S (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30:2725-2729.

Thompson RC and McManus DP (2002). Towards a taxonomic revision of the genus *Echinococcus*. Trends in Parasitology 18:452-457.

Wahlers K, Menezes CN, Wong ML, Zeyhle E, Ahmed ME, Ocaido M, Stijnis C, Romig T, Kern P and Grobusch MP (2012). Cystic echinococcosis in sub-Saharan Africa. Lancet Infectious Diseases 12:871-880.

News

Genetics project focussed on camel beauty



Dr Jaime Gongora, a scieintist of wildlife and animal genetics and genomics at The University of Sydney, and his PhD student Mahmood Al Amri are developing an exhaustive scorecard for camel beauty contests. The scorecard will look at morphometric measurements, which are those that relate to the shape and dimensions of the parts of a camel's body assessed during judging. Genetic studies of the camels will be linked to the development of the scorecard which would allow the researchers to carry out an "association analysis" between the physical, or phenotypic, traits of individuals, and their genetic makeup. Two main types of markers, i.e. microsatellites, which are sections of repeated DNA sequences that vary from one animal to the next and Random Amplified Polymorphic DNA (RAPD) markers, in which random sequences of DNA are used for comparison.

(Source: The National, UAE)