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Molecular and morphological characterisation of *Echinococcus* from food producing animals in India

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ABSTRACT

In view of the medical, veterinary and economic importance of hydatid disease in India, our study aimed to determine the prevalence and genotypes of *Echinococcus* present in domestic livestock in India. Out of 21,861 animals examined, cattle were found with the highest prevalence of hydatid cysts (5.10%) followed by buffaloes (3.81%), pigs (0.87%) and sheep (0.075%). Phylogenetic analysis of the cytochrome oxidase-1 gene revealed that the buffalo strain or G3 genotype was the predominant genotype (29/46) in all species of livestock followed by the cattle strain or G5 genotype (9/46), the G1 genotype or the common sheep strain (6/46) and the G2 genotype or Tasmanian Sheep strain (2/46). The ability of the G3 (buffalo) and G5 (cattle) genotypes of *Echinococcus granulosus* to infect and produce fertile hydatid cysts in pigs was also demonstrated for the first time. Both morphological and molecular results support earlier studies suggesting that *Echinococcus* of buffalo origin is phenotypically and genetically similar to the sheep (G1) and Tasmanian Sheep (G2) strains of *Echinococcus*, which adds further evidence to support its recognition as one species, *viz.*, *E. granulosus* sensu stricto. Our molecular, morphological and biological characteristics also support earlier studies suggesting that *Echinococcus* of cattle origin, designated the G5 genotype, should be recognised as a separate species, *viz. Echinococcus ortleppi*. Finally, the study reveals that the prevalence of hydatidosis in urban centres in India has been showing a consistently declining trend over the past few decades, possibly owing to economic development and improved government legislation of abattoirs.

1. Introduction

Cystic echinococcosis, a common metacestode infection in food producing animals also poses a major public health problem, especially in developing countries. Humans are infected with hydatid cysts during natural transmission of the disease from carnivores to domestic animals, by accidentally consuming eggs of *Echinococcus granulosus* through contaminated food, water and soil, or through direct contact with dogs. Although the disease in domestic animals is usually asymptomatic and detected only at the time of post-mortem inspection at the abattoir, it causes great economic loss through condemnation of infected offal, in particular, liver. In 2005, the contribution of the Indian livestock industry to the GDP was 6.8% and in 2002 India exported US $320.4 million worth of meat and edible meat offal (FAO, 2005). Previous surveys of hydatid disease in food producing animals in India have revealed that the disease is endemic throughout the country (see Table 1). Measures to control hydatid diseased would not be beneficial the Nation's economy, but also human health. Over 500 cases of hydatid disease requiring surgery has been sporadically reported in the human medical literature of India within the last 50 years (Traub et al., 2005). In

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Table 1
A summary of published literature on the prevalence of hydatid disease in livestock (expressed as a range) in different geographical locations in India.

Region	Host	Prevalence (%)			References
		1980–1990	1991–2000	2001 onwards	
North	Cattle	7.8	21.9	Information not available	Deka et al. (1983), Dhar and Singh (1995)
	Buffalo	11.3–48.1	18.39	Information not available	Singh and Dhar (1988), Deka and Gaur (1990), Varma and Malviya (1992)
	Sheep	4.7–30.5	2.56–7.2	Information not available	Singh and Dhar (1988), Varma (1990), Jithendran (1996), Deka and Gaur (1998)
	Pig	1–11.25	0.73–1.42	Information not available	Irshadullah et al. (1989), Singh et al. (1988), Varma and Malviya (1992), Deka and Gaur (1998)
South	Cattle	1.7–42.12	6.37–11.85	14.8	Prabhakarn et al. (1980), Reddy et al. (1983), Vijayasmitha et al. (1993), Hafeez et al. (1994), Balamurugan et al. (2003)
	Buffalo	4.0–22	7.24–9.8	7.3	Reddy et al. (1983), Kulkarni et al. (1986), Vijayasmitha et al. (1993), Shanumugam et al. (1994), Balamurugan et al. (2003)
	Sheep	2.5–9.7	3.7–47.6	8.92	Abraham et al. (1980a,b), Kulkarni et al. (1986), Murlidharan and Sastry (1996), Das and Sreekrishnan (1998), Balamurugan et al. (2003)
	Pig	0.0	3.02–6.89	Information not available	Reddy et al. (1983), Vijayasmitha et al. (1993), Hafeez et al. (1994)
East	Cattle	17.8–31.9	13.3–45	16.76–21.43	Sanyal and Sinha (1983), Biswas et al. (1989), Das and Das (1998), Sharma et al. (2000), Deka et al. (2008)
	Buffalo	42.25	27.6–48	6.52	Biswas et al. (1989), Das and Das (1998), Sharma et al. (2000), Deka et al. (2008)
	Sheep	8.3–50	9.0	Information not available	Prasad and Prasad (1980), Katiyar and Sinha (1981)
	Pig	7.6	1.79–8.0	0.34–0.43	Prasad (1981), Sharma et al. (2000), Deka et al. (2008).
West	Cattle	4.2–21.6	4.16–21.8	13.17	Kulkarni et al. (1984), Gatne et al. (1989), Dhote et al. (1992), Munde (1999), Gatne (2001)
	Buffalo	Information not available	4.6	34.5	Munde (1999), Khan and Purohit (2006)
	Sheep	Information not available	0.2	0.85	Munde (1999), Gatne (2001)
	Pig	Information not available	0.21	3.14–5.58	Gatne (2001), Gaurat and Gatne (2005)

India, ideal conditions exist for the establishment, propagation and dissemination of cystic echinococcosis in both humans and livestock. A lack of education and knowledge about the life cycle of the parasite and the lack of veterinary meat inspection and offal disposal at illegally run abattoirs significantly contributes to domestic cycles of transmission. Moreover, home-slaughter, especially for religious events or in rural communities, is commonly practiced throughout the country, and stray and semi-domesticated dogs are given ample opportunity to be exposed to infection.

To date, nine genotypes (G_1–G_{10}) of *E. granulosus* have been identified using molecular tools and the strain variation closely follow the parasite's biological and phenotypic characteristics (McManus and Thompson, 2003; Nakao et al., 2007). Recently it has been proposed that *E. granulosus* may be a species complex which are likely to be maintained in distinct cycles of transmission comprising of *E. granulosus* sensus stricto (genotypes G1–G3), *Echinococcus equinus* (genotype G4), *Echinococcus ortleppi* (genotype G5), G6/G7, *Echinococcus canadensis* (genotypes G8 and G10) and *Echinococcus felidis* ('lion strain') (Nakao et al., 2007; Huttner et al., 2008). Studies correlating morphological criteria based on the metacestode rostellar hook dimensions with genotype have provided further support for this hypothesis (Thompson et al., 2006). To date, no data on the morphological characteristics of the Indian Buffalo strain (G3) of *Echinococcus* exists to support its proposed placement within the G1/2/3 cluster.

Barring the report published by Bhattacharya et al. (2006) and more recently, Gudewar et al. (2009), who found isolates of *E. granulosus* belonging to genotypes G1, G2 and G3 from livestock in West Bengal, a detailed investigation on the genotypes of *E. granulosus* within a larger geographical area of India, has yet to be performed. In view of the medical, veterinary and economic importance of hydatid disease in India, our study aims to ascertain the prevalence and molecular epidemiology of hydatid disease in food producing animals in India by genetically and morphologically characterising hydatid cysts recovered from a range of domestic livestock, namely cattle, buffalo, sheep and pigs. From a practical point of view, the recognition of strain variation is a major prerequisite for strategic control efforts aimed at limiting transmission in endemic area.

2. Materials and methods

2.1. Sampling design and collection of hydatid material

Between January 2007 and February 2008 a total of 21,861 animals, including 824 cattle, 1050 buffaloes,