National Centre for Animal Health, Bhutan

Universität Zürich[UZH]

IPZ Institute of Parasitology University of Zurich Switzerland

Identification of *Echinococcus* spp. in definitive and intermediate hosts in Bhutan

[1]Thapa, N. K. ,[1]Sharma P.M., [1]Lham, S; [1]Dema, T; [1]Kinzang, D.,[1]Gurung, R. B. and [2]Deplazes, P.
[1]National Centre for Animal Health, Serbithang, Thimphu, Department of Livestock, Bhutan, [2]Institute of Parasitology, University of Zurich, Zurich, Switzerland

Introduction

Earlier pilot studies indicated the presence of *Echinococcous* spp. in community/stray dogs and cystic echinococcosis (CE) in intermediate hosts in Bhutan. Records from Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) also revealed the occurrence of CE in humans between the years 2006-2013 (Pelden 2013). However no epidemiological studies have been conducted to identify *Echinococcus* spp. prevalent in community/stray dogs and in intermediate hosts.

Community/stray dogs in Bhutan

Materials & Methods

Through environmental sampling, 138 dog fecal samples collected from community/stray dogs from Thimphu, Gelegphu and Tsirang areas and also 28 fecal samples collected from the forest area near Mithun breeding farm from the central region were analyzed for the presence of taeniid eggs by the floatation and sieving method (F/Si) and by multiplex PCR (Trachsel *et al.* 2007).

In total, 55 cyst samples were collected from slaughterhouses (Tsirang), yak slaughter places (Thimphu, Paro, Haa) and imported beef carcass from checkpoints (Phuntsholing, Gelegphu, S/jongkha) in 2011-2012. The fertility status' of the cysts were determined by the presence of protoscoleces (PSC) in the hydatid fluid. The cysts without PSC as well as suppurated or calcified lesions were considered as infertile. The sediment containing PSC or germinal layer was collected and subjected to molecular diagnosis.

Molecular analysis

Genomic DNA was extracted from each sample (n=58) using the tissue protocol described in the Qiagen mini kit (Qiagen, Hilden, Germany). All samples were subjected to PCR targeting two mitochondrial genes, NADH dehydrogenase subunit 1 (nad1) and cytochrome oxidase subunit 1 (cox1) using the primers and the conditions described by Bart *et al.* (2006).

In order to assess the species level for *Taenia* spp. (fecal samples) and *Echinococcus* spp. (cyst samples), the amplicons of each positive sample were sent for direct sequencing, after purification using the MinElute PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions (for further details see Thapa *et al.* 2017).

Table 1. Identification of *Echinococcus* spp. in fecal samples of canids

Sample origin	Taeniid positive	Multiplex – PCR findings	Sequence findings
Stray or community dogs (n=138)	20	16 non-*Echinococcus* cestodes	1 *T. hydatigena*, 9 *Spirometra* sp., 6 not sequenced
		10 *E. granulosus* sensu lato (8 mixed infection with non-*Echinococcus* cestodes)	Not further characterized
		2 negative	
Forest area surrounding Mithun farm (n=28)	14	8 non-*Echinococcus* cestodes	6 *T. hydatigena*, 1 *H. taeniaeformis*, 1 not sequenced
		1 *E. granulosus* s.l.	Not sequenced
		6 negative	

Results & Discussion

Table 1 depicts the findings of the microscopic examinations of the fecal samples, Multiplex-PCR and sequencing analysis and Table 2 shows the findings of the cyst samples.

E. granulosus s.l. has been identified in the community/stray dogs in Bhutan which could be due to improper slaughter practices of the animals. Similar findings have been reported from other parts of Asia including northern India and Nepal (Reece 2005).

From the cysts, *Echinococcus granulosus* (G1–3) was the prominent species found, the findings are in accordance with earlier studies conducted in Nepal (Joshi *et al.* 1997). In Bhutan, sheep is reared for mainly wool purposes in small numbers. Therefore, samples could not be collected from sheep to confirm a dog sheep cycle. However, the presence of *E. ortleppi* in cattle indicates the existence of a dog cattle cycle in Bhutan. Cyst samples from humans will be analyzed in the near future and further studies are required to elucidate the epidemiology of *Echinococcus* in Bhutan.

A Yak (*Bos grunniens*) from Bhutan

Table 2. *Echinococcus* spp. identified in cysts (n=54) from cattle or yaks

Origin	Infectivity of cysts	*E. granulosus* (G1-G3)	*E. ortleppi* (G5)
Imported beef (India, Nepal)	Fertile	4	1
	Sterile	31	0
Cattle slaughtered in Bhutan	Fertile	1	1
	Sterile	6	0
Yaks slaughtered in Bhutan	Fertile	0	0
	Sterile	10	0

Acknowledgement

I acknowledge the International foundation for Science (IFS) Sweden, for providing the research grant for conducting this study. My sincere thanks to the Institute of Parasitology, University of Zurich for guiding the study and also for providing funding support to attend this conference. Lastly I'm highly indebted for the support provided by WAAVP 2017 for students of south east Asian countries.

References

J.M. Bart, S. Morariu, J. Knapp et al. (2006) Genetic typing of Echinococcus granulosus in Romania. Parasitol. Res., 98, pp 130-137.

D.D. Joshi, A.B. Joshi, H. Joshi, Epidemiology of echinococcosis in Nepal, Southeast Asian J. Trop. Med. Public Health 28 (1997) 26–31.

J.F. Reece, Dogs and dog control in developing countries, in: D.J. Salem, A.N. Rowan(Eds.), The State of the Animals III, Humane Society Press, Washington DC 2005,pp. 55–64.

S. Pelden. Retrospective study on echinoccosis in humans in Bhutan. Proceedings of the National One Health Symposium held during 18- 20 November (2013) at Phuntsholing Bhutan

N.K. Thapa, M.T. Armua-Fernandez ,D. Kinzang, R.B. Gurung, P. Wangdi , P. Deplazes (2017) Detection of Echinococcus granulosus and Echinococcus ortleppi in Bhutan. Parasitol Inter. 66, 139-141.

D. Trachsel, P. Deplazes, A. Mathis (2007) Identification of taeniid eggs in the feces from carnivores based on multiplex PCR using targets in mitochondrial DNA, Parasitology 134 (2007) 911–920.