

## A PCR Analysis of Prevalence of *Echinococcus granulosus* Genotype G1 in Small and Large Ruminants in Three Districts of Punjab, Pakistan

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**Abstract.-** Prevalence of *Echinococcus granulosus* genotype G1 was investigated in 55 cattle, 129 buffaloes, 10 sheep and 10 goat samples by polymerase chain reaction (PCR). These 204 samples were collected from 64 liver and 140 lungs of slaughtered animals from three districts Okara (n= 29), Jhang (n = 43) and Lahore (n= 132). *E. granulosus* G1 genomic DNA was used for specific amplification of a 254-bp fragment. Twenty five (45.45%) cattle, 78 (60.46%) buffaloes, 2 (20%) sheep and 2 (20%) goat samples were positive for *E. granulosus* genotype G1. Twenty four (37.5%) liver and 80 (57.14%) lung samples were found positive. The prevalence of *E. granulosus* genotype G1 was 37.93 % in district Okara, 65.11 % in district Jhang and 50 % in district Lahore. These results indicate that the PCR is valid and sensitive for surveying the disease in epidemiological studies.

**Keywords:** *Echinococcus granulosus*, PCR, zoonosis, hydatid cyst.

### INTRODUCTION

Pakistan has a large livestock population, well adapted to the local conditions and has some of the best tropical dairy breeds (Afzal and Naqvi, 2004). Current livestock-population (2013-2014) of the country include 34.6 million (M) buffaloes, 39.7 M cattle, 29.1 M sheep, 66.6 M goats and 1.0 M camels (Anonymous, 2014). These produce 50.990 M tones of milk, 1.887 M tonne of beef, 0.657 M tones of mutton, 44.1 thousand tonne of wool and 66.74 M skins and hides (Anonymous, 2014).

Despite such a heavy population of livestock in Pakistan, the production by these animals is not as much as it should be. Poor breeding selection, management deficiencies, and prevalence of many parasites such as *Echinococcus granulosus*, affects the livestock productivity (Bhattacharya *et al.*, 2008a). This parasite has dogs and other canids as definitive hosts, and herbivorous, omnivorous species including wildlife and domesticated livestock as intermediate hosts (Latif *et al.*, 2010). The larval stage of *E. granulosus* is a fluid filled bladder or hydatid cyst that is unilocular, although

communicating chambers also occur. Individual bladder may reach up to 30 cm in diameter and occur most frequently in liver and lungs but may develop in other internal organs. The infection with this stage is referred to as cystic echinococcosis (OIE, 2008).

A number of distinct strains of *E. granulosus* have been described and till date, 10 different genotypes (G1 to G10) have been identified using molecular tools (Latif *et al.*, 2010). The G1 sheep strain of *E. granulosus sensu lato* is considered as the most common and frequent cause of disease in humans and animals (OIE, 2011). In one study economic losses due to *E. granulosus* in livestock per 100 sheep and goats were estimated US\$276.20 and for 100 infected buffaloes, cattle and camels were US\$165.72 (Latif *et al.*, 2010). Economic losses including low quality and less production of milk, meat or wool, retarded growth, low fertility and condemnation of infected organs. To date, a detailed investigation on the genotype of *E. granulosus* in livestock has yet to be performed in view of medical, veterinary and economic importance of hydatid disease in Punjab, Pakistan. Therefore, this study was initiated to investigate the prevalence, molecular diagnosis and localization of most commonly prevalent genotype G1 of *E.*

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*granulosus* in three heavily livestock populated districts of Punjab by using polymerase chain reaction (PCR).

## MATERIALS AND METHODS

A total of 204 samples comprising of fertile cysts from different parts of body such as liver, lungs, etc., were collected from cattle, buffalo, sheep and goats, slaughtered at different abattoirs in districts Okara (n=29), Jhang (n=43) and Lahore (n=132) from December 2012 to April 2013. Animals of all age groups and both sexes were included in the study. The whole cyst samples were collected without rupturing the sterile bags and brought to lab in ice containers and stored at -20°C till further processing and DNA extraction for molecular characterization at Livestock Production Research Institute Bahadurnagar, Okara, Punjab Pakistan.

Protoscolices or cyst wall (germinal and laminar layers) (0.5 g) were excised by using sterile scissor or hydatid fluid was withdrawn from the cyst by sterile needle syringe (Dinkel *et al.*, 2004; Bhattacharya *et al.*, 2008b) and then subjected to total genomic DNA isolation by using TRIAGENT<sup>®</sup> according to the manufacturer's instructions. The nucleic acid was extracted also from known negative blood samples (20 healthy buffaloes maintained at Livestock Production Research Institute Bahadurnagar Okara). The number of genomic copies were determined by using Minerva Biolabs kit "Venor Gem<sup>®</sup> and the samples were stored at -20°C till further use. The primers described by Dinkel *et al.* (2004), targeting the mitochondrial 12S rRNA gene, consisted of forward primer E.g.ss1for. (5'-GTA TTT TGT AAA GTT GTT CTA-3') and reverse primer E.g.ss1rev. (5'-CTA AAT CAC ATC ATC TTA CAA T-3') were used as *E. granulosus* genotype G1-specific primers for amplification of 254-bp fragments from *E. granulosus* G1 genomic DNA. All PCR amplification reactions, including control negative samples were carried out in a final volume of 25 µl containing 5 µl lysate or 2 ng purified DNA as DNA template, 12.5 µl commercially available PCR master mix (Pyro Start<sup>™</sup> Fast PCR Master Mix-2X, Fermentas, Ontario, Canada), 2 U *Taq* DNA

polymerase (Thermoscientific, California, USA) and 2 Mm MgCl<sub>2</sub> (Thermo scientific, California, USA). The primers were used at the concentration of 50 pmol µl<sup>-1</sup>.

PCR was carried out in a DNA thermal cyclor (PEQLAB Biotechnologie, GmbH, Erlangen, Deutschland) at the following parameters: Initial denaturation for 30 s at 94°C then 40 cycles with each 30 s denaturation at 94°C, 60 s annealing at 57°C and amplification of 40 s at 72°C and final extension for 5 min at 72°C (Dinkel *et al.*, 2004). Twelve microliters of the PCR products admixed with 6x DNA loading dye (Fermentas<sup>™</sup>, Ontario, Canada) were sized by electrophoresis on a 1% agarose gel (Gene choice<sup>®</sup>, Maryland, USA) (1 h at 90 V) with 100- bp ladder (Fermentas<sup>™</sup>, Ontario, Canada) as size marker. The gels were stained with ethidium bromide (HP 47.1, ROTH, Karlsruhe) (250 µg/ml @ one drop/25 ml of 1 % agarose gel) and analyzed in a UV trans-illuminator (Dolphin -Doc, Wealtec, NV, USA) for visualization of PCR products.

## RESULTS

One hundred five (51.47 %) out of 204 *E. granulosus* infected samples showed amplification of a 254-bp fragment (Fig. 1). No such amplicon was detected in control negative samples.

District wise details of prevalence of *E. granulosus* G1 genotype has been shown in Table I. The P-value (0.1418\*) of the calculated ( $\chi^2$ ) statistic value is greater than the level of significance *i.e.*,  $\alpha$ : 0.10; hence the proportional prevalence of *E. granulosus* G1 genotype is homogenous in different districts.

**Table I.** Prevalence of *E. granulosus* genotype G1 in different districts of Punjab, Pakistan

Districts	Total number of samples	Positive samples	Prevalence %
Okara	29	11	37.93 <sup>a</sup>
Jhang	43	28	65.11 <sup>a</sup>
Lahore	132	66	50.00 <sup>a</sup>
Total	204	105	51.47

<sup>a</sup> Since ( $\chi^2$ ) statistic value (=5.45) has P-value (=0.1418) >  $\alpha$ : 0.10 and therefore the proportional prevalence of *E. granulosus* genotype G1 is homogenous in different districts.

**Table II.- Prevalence of *E. granulosus* G1 genotype in different organs**

Districts	Liver			Lung		
	Total no of samples	Positive samples	Prevalence %	Total no of samples	Positive samples	Prevalence %
Okara	11	6	54.54	18	5	27.77 <sup>a</sup>
Jhang	15	5	33.33	28	23	82.14 <sup>a</sup>
Lahore	38	13	34.21	94	52	55.31 <sup>a</sup>
Total	64	24	37.50	140	80	57.14

<sup>a</sup> Since ( $\chi^2$ ) statistic value (=6.31) has P-value (=0.0427\*\*) <  $\alpha$ : 0.05 and thus concluded that the lungs has significantly greater proportions of prevalence of *E. granulosus* genotype G1 rather than liver.

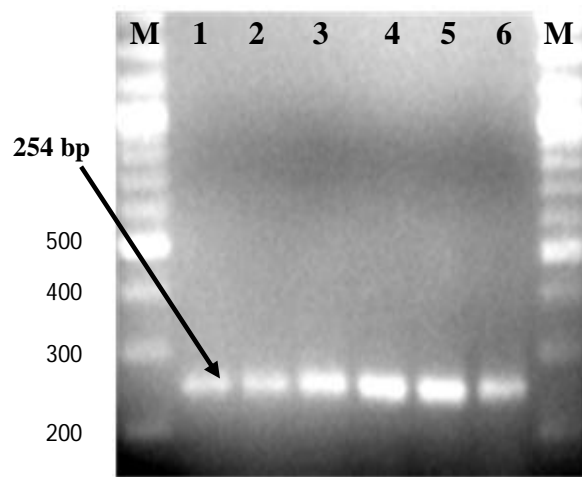


Fig. 1. Confirmation of *Echinococcus granulosus* genotype G1 from sheep (Lane 1), goat (Lane 2), cattle (Lanes 3 and 4) and buffalo (Lanes 5 and 6) cyst samples by PCR by amplification of six field samples with primer pair E.g.ss1for. and E.g. ss1rev. at~ 254- bp. Lane M: Molecular ladder; Lanes 1, 2, 3, 4,5, 6: test samples + ve for *Echinococcus granulosus* genotype G1.

Similarly, the prevalence percentage of *E. granulosus* G1 genotype in different organs has been shown in Table II. Since the P-value (0.0427\*\*) of the calculated ( $\chi^2$ ) statistic value is less than the level of significance *i.e.*  $\alpha$ : 0.05 and thus concluded that the lungs has significantly greater proportions of prevalence of *E. granulosus* G1 genotype rather than liver.

The prevalence of *E. granulosus* G1 genotype in different species of animals has been shown in Table III. Since the P-value (0.0121\*\*) of the calculated ( $\chi^2$ ) statistic value is less than the level of significance *i.e.*  $\alpha$ : 0.10 and thus concluded that the

proportional prevalence of *E. granulosus* G1 genotype is significantly different among different species, further buffaloes and cows have higher prevalence of *E. granulosus* G1 genotype rather than sheep and goats.

**Table III.- Prevalence of *E. granulosus* G1 genotype in different species of animals.**

Species	Total no of samples	Positive samples	Prevalence %
Cattle	55	25	45.45 <sup>a</sup>
Buffalo	129	78	60.46 <sup>a</sup>
Sheep	10	2	20 <sup>a</sup>
Goat	10	2	20 <sup>a</sup>
Total	204	107	52.45

<sup>a</sup> Since ( $\chi^2$ ) statistic value (=12.85) has P-value (=0.0121\*\*) <  $\alpha$ : 0.10 and thus concluded that the proportional prevalence of *E. granulosus* genotype G1 is significantly different by different species, particularly buffaloes and cows have higher prevalence rather than sheep and goats.

## DISCUSSION

In Pakistan, the prevalence of hydatidosis has been reported in different breeds of animals such as cattle, buffaloes, sheep, goats and camels. Iqbal *et al.* (2012) has observed prevalence of hydatidosis in sheep (8.85%) and in goats (6.21%) in Lahore district while Ahmad *et al.* (2006) has reported 46.74% hydatidosis in sheep in Quetta . Similarly, Latif *et al.* (2010) have observed the prevalence of *E. granulosus* in camel (17.29%), buffaloes (7.19%), and cattle (5.18%). The findings of our study are in accordance with the above mentioned research findings as we observed the prevalence of *E. granulosus* genotype G1 in cattle (45.45%), buffalo (60.46%) sheep (20.00%) and goats

(20.00%). Latif *et al.* (2010) have reported 5.18% prevalence in cattle, 7.19% in buffalo, 7.52% in sheep and 5.48% in goat during 2004-2008. During this study 107 out of total 204 samples were found positive for *E. granulosus* genotype G1. The negative samples were probably positive for the *Echinococcus granulosus* for some other genotype of the parasite.

In the study a higher prevalence of *E. granulosus* was observed in liver (38.09%) and lungs (57.14%), as compared to Iqbal *et al.* (2012) who observed prevalence in liver (33.91%) and in lungs (45.19%) in Lahore district in sheep and goats. The high prevalence in the present study cannot be compared with the Iqbal *et al.* (2012) research findings because that study was conducted in only one district, while the present study had been conducted in three districts.

This PCR can be suitable to be implied for copro-diagnosis. Further, species and genotype specific diagnosis of *E. granulosus* infection in final hosts, which represent direct or indirect source of human infection, is the most reliable indicator for the potential risk for human (Schantz *et al.*, 1998). Hence it is a source of vital information about risk factor for human cystic echinococcosis, especially in developing countries like Pakistan, where human and livestock infection is spreading.

In conclusion PCR for the detection of *E. granulosus* G1 genotype for cattle, buffalo, sheep and goats is specific and sensitive. The test is suitable for the epidemiological surveys in slaughtered animals, which would be useful for designing hydatidosis control program in endemic areas.

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