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RESTRICTION DIGESTION ANALYSIS OF GENOMIC DNA OF THE CESTODE PARASITE, *Raillietina Tetragona* INFECTING THE INTESTINE OF THE COUNTRY FOWL (*Gallus Domesticus)*

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ABSTRACT

Economic losses due to helminth parasites in country fowl throughout the world are considerable. Though less emphasis has been placed on utilising DNA elements to study cestodes of veterinary importance, correct diagnosis is, nevertheless, critical to proper treatment and control. These are able to induce structural, biochemical and immunological changes in the host like inappetence, diarrhoea, dull hair coat and ostertagiosis. At present, control is almost exclusively based on anthelmintics. Hence, Genomic DNA analysis plays an increasingly important role in characterising and classifying cestode parasites. The present study revealed restriction digestion analysis of genomic DNA of *Raillietina tetragona* reference isolates collected from country chicken "*Gallus domesticus*" from Zambazaar market, Triplicane, Chennai (Tamil Nadu) using 4 restriction enzymes revealed some differences in the restriction profile of DNA in the chick isolates. Bgl I and Pvu II restriction digestion showed bands above 23Kbs of Lambda Hind III marker and streak formation below 2Kbs. Dra I showed a single band above 23 Kbs of lambda Hind III marker. No restriction site and bands could be fixed unambiguously with restriction enzyme Hinf I.

KEYWORDS: Country chicken, *Raillietina tetragona*, Genomic DNA analysis, Restriction enzymes.

INTRODUCTION

Molecular approaches are the most effective and accurate means for the detection of many organisms and for screening of genetic variation among populations (Wongsawad and Wongsawad, 2010). Economic losses due to helminth parasites in sheep throughout the world are considerable (Irfan-ur-Rauf Tak *et al*., 2013). The features of the host – parasite relationships among the taeniid cestodes have formed the basis for vaccine development. Research on the development of vaccines to prevent infection with cestode parasites has brought focus on the taeniid cestodes (Lightowlers *et al*., 2002). *Raillietina tetragona* belonging to the family of *Davaineidae* are of common occurrence. According to Southwell (1930) *Raillietina* species are considered quite serious pathogens. Older birds Capillary congestion, cellular infiltration and connective tissue proliferation in intestinal wall and fibrosis are other conditions which have been associated with tapeworm infection (Nath and Pande, 1963). Studies and reports have revealed that among the diverse species of *Davaineidae* cestode parasites that inhabit the intestine of fowl *R. tetragona* is the most harmful one, as appraised by the pathological implications.

The tapeworm *Raillietina* species is one of the most pathogenic parasites which occur in the small intestine of chicken and turkeys in most parts of the world (Ramesh Kumar *et al.*, 2007). Nodular disease caused by the cestode, *Raillietina* species is widely recognized as an increasingly important disease in chicken in developing countries. Nodules and hyperplastic enteritis may develop at the site of attachment in the intestine of chicken. This phenomenon is named “nodular tapeworm disease” and may occur in heavy infections. Infections with *R. tetragona* are found in chicken and turkeys. It is cosmopolitan in distribution (Anders Permin and Hansen (1999).

The presence of *R. tetragona* cystic nodular forms of the parasite on the serosa of the intestinal tract, and lungs, can interfere with the normal functioning of the organs in chicken (Ashenafi and Eshetu, 2004). The pathological changes in the intestines of domestic fowls were characterized by villous atrophy, enteritis with cellular infiltration and formation of characteristic granulomas as reported by Samad *et al*., (1986). *R. tetragona* is most harmful among *Davaineidae* cestodes (Yamaguti, 1959). The present study reports the results of restriction digestion analysis of genomic DNA of chick isolate *R. tetragona* with the restriction endonuclease enzymes Bgl I, Pvu II, Dra I and Hinf I which belongs to type II restriction endonuclease enzyme family. Type II Restriction endonucleases recognize short,

usually palindromic sequences of 4–8 bp and, in the presence of Mg^{2+}, cleave the DNA within or in close proximity to the recognition sequence (Alfred Pingoud and Albert Jeltsch, (2001).

Bgl I is a type II restriction enzyme. The active form of Bgl I restriction enzyme is a single polypeptide with a molecular weight of 32,000. Bgl I is not sensitive to sulfhydryl reagents but gets affected by reagents that modify lysine and arginine residues (Lee and Chirikjian, (1979). Pvu II was isolated from *Proteus vulgaris.* Pvu II is one of the type II restriction endonucleases. This enzyme recognizes a common two – fold symmetrical hexanucleotide sequence in duplex DNA to make blunt end (Jong – In Lee and Chul – Hak Yang, 1984). A type II restriction endonuclease, Dra I, isolated from *Deinococcus radiophilus* recognizes the palindromic hexanucleotide sequence. Ultraviolet irradiation of the DNA substrate at relatively low doses inhibits the activity of Dra I by "protecting" the recognition sequence and this may be exploited to give control of partial digestion of DNA by Dra I (Purvis and Moseley, 1989). Hinf I restriction endonuclease was isolated from *Haemphilus influenzae*. The genes coding for Hinf I restriction and modification enzymes were cloned from the *Haemophilus influenzae* Rf strain using pUC18 plasmid as vector (Arlene Rodriguez *et al.*, 1995).

MATERIALS AND METHODS

Animal procurement (Parasite preparation)

The cestode parasite, *Raillietina tetragona* (Molin 1858) were obtained alive from the intestine of naturally infected and recently killed country fowl "*Gallus domesticus*" collected from Zambazaar market, Triplicane, Chennai (Tamil Nadu). Then the collected specimens were rinsed in distilled water to render them free from intestinal contents and kept in separate petridishes for identification. The entire worms were spread out on a glass pane and the length of the worms was measured. The immature, mature and gravid proglottid regions of both the worms were identified and separated and were homogenized with 2ml of distilled water and used for biochemical analysis. Restriction enzymes - Bgl I, Pvu II, Dra I and Hinf I were used for restriction digestion analysis of Genomic DNA of the cestode Parasite, *Raillietina tetragona* infecting the intestine of the country chicken (*Gallus domesticus)*.

Ahmed and Abdel-Moaty (2011) reported that *Cotugnia polycantha* infecting doves, *Streptopelia senegalensis*, differs from that infecting pigeons, *Columba livia domestica*. Sperm ultrastructure was used as a new tool for identification and *C. Polycantha* infecting

two different hosts, doves *S. senegalensis* and pigeons *C. livia domestica*, were compared using random amplified polymorphic DNA (RAPD) analysis for differentiation between them. In the present study the genomic DNA of two cestode parasites were studied using multisource genomic DNA, mini-prep kit and electrophoretic study by Agarose gel electrophoretic method (Sabry E. Ahmed, 2012).

Genomic DNA isolation

Genomic DNAs were isolated on a small scale from 1ml of adult worms using multisource genomic DNA, Mini-Prep Kit, Axgene Biotechnology, U.S.A Cat. No. 110420 - 25, according to manufacture manual. Gene extraction studies do not reveal protein turnover patterns and protein modifications that may be involved in signalling and communication, protein transport and targeting important phenomenon (Savithry Natarajan *et al*., 2014). The isolation of total genomic DNA from *R. tetragona* was carried out under two steps:

Extraction of genomic DNA

10.1 grams of the sample of the parasite was weighed and mixed well with 10ml of lysis (NaCl, Tris HCl (pH 8.0), EDTA, SDS) buffer till the tissue was blended with the buffer. The tissue was left overnight at room temperature. 5ml of saturated phenol and 5ml of saturated chloroform were added and mixed well. The tube was gently inverted to mix the aqueous and organic phases until a cloudy emulsion was formed. The tube was then centrifuged at 12000 rpm for 15 minutes to separate the phases. After centrifugation the upper aqueous phase containing the DNA and the lower organic phase, containing the denatured protein and other contaminants were observed. The aqueous phase with DNA was transferred to a 1.5ml tube and the organic phase was discarded.

Precipitation of genomic DNA

1.1ml of 3m sodium acetate (pH 4.8) was added to 11ml of DNA solution. The tube was capped and inverted to mix well and the DNA was precipitated with the help of 25ml of absolute ice cold ethanol. The tube was centrifuged at 12,000 rpm for 15 minutes and the supernatant was discarded. The pellet of DNA was washed with 70% ethanol and centrifuged at 12,000 rpm once again for 15 minutes. The supernatant was discarded and the precipitated DNA was removed until the excess ethanol has drained away and evaporated. Then TE buffer was added and the agarose gel electrophoresis was carried.

Restriction endonuclease enzyme digestion

Genomic DNA of *R. tetragona* was digested with restriction endonuclease enzymes using reaction conditions of the enzymes. The restriction enzymes tested were Bgl I, Pvu II, Dra I and Hinf I. A typical enzyme reaction consisted of 2 micrograms of genomic DNA, 5 microlitres of restriction buffer, 0.4 microlitres of each restriction enzyme and 4.6 microlitres of sterile water. The enzymatic reaction was incubated using a water bath at 37^0C for 35-45 minutes.

Agarose gel electrophoresis

Agarose gel Electrophoresis was carried out according to the procedure as outlined by Sambrook and Russel (2001). The DNA analysis of the cestode samples was started by preparing a 0.7% agarose gel to run the samples. TAE was used as the buffer with a concentration of 40mM tris (pH7.6), 20mM acetic acid and 1mM EDTA was used as the marker gene. Bromophenol blue was used as the marker dye at a concentration of 1µl for every 5µl of DNA sample. Ethidium bromide was used as the visualization dye which was mixed in the buffer tank at 0.5mg/ml. The solution was well stirred to disperse ethidium bromide. 20µl of DNA sample were loaded into lane 1 and the marker gene λ Hind III sample was loaded into lane 2. The gel electrophoresis was carried out separately for the two cestode parasites. Current was supplied typically at 100V for 30min. After electrophoresis the gels were observed under a UV lamp wearing a protective gear.

UV – Spectrophotometer for sample purity

The double beam UV-Spectrophotometer (Shimadzu Corporation) connected to PC UV1650 was used for sample testing and all further operations were done using computer keys. Thus by using UV spectrophotometer the sample DNAs of the cestodes, *Raillietina tetragona* were assessed for their purity, and the ratios of pure preparations of DNA of both the samples were found to be close to 2.0.

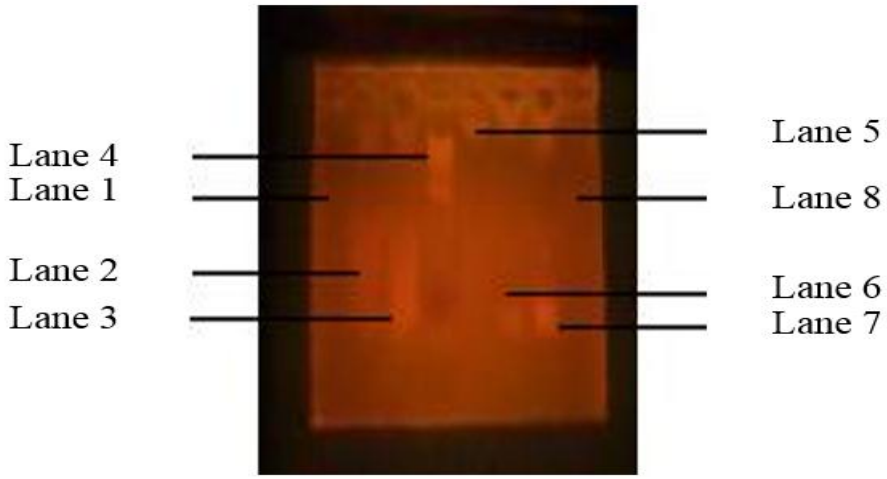
RESULTS AND DISCUSSION

The restriction profiles of DNA from *R. tetragona* reference isolate from country chicken are shown in fig: 1. *R. tetragona* DNA from the referral chick isolate could be restricted by Bgl I, Pvu II and Dra I. While no restriction site could be fixed with the restriction enzyme Hinf I. Restriction digestion of genomic DNA of *R. tetragona* with the restriction enzymes Bgl I and Pvu II formed a band above 23 Kbs of Lambda Hind III marker and streak formation below 2Kbs. Whereas, restriction digestion with Dra I formed a single band which was above 23

Kbs of Lambda Hind III marker. Restriction digestion of genomic DNA of *R. tetragona* with the restriction endonuclease enzyme Bgl I showed a small light band above 23kbs of Lambda Hind III marker and streak formation below 2kbs. But in the case of cattle isolate *Echinococcus granulosus,* DNA digested with Bgl I fixed no restriction site as reported by Reddy and Rao, (1998).

Restriction digestion of genomic DNA of *R. tetragona* with the restriction endonuclease enzyme Hinf I showed no restriction sites. Whereas Reddy and Rao, (1998) reported 3 restriction sites in *E. granulosus* of buffalo isolates as 3.4, 2.2 and 2.1 kb DNA bands within the smear of DNA with Hinf I and cattle DNA showed uniform smearing with low resolution. In the present investigation, studies on cestode DNA macromolecule of *R. teragona* provide the basic understanding in the isolation of genomic DNA and restriction digestion of DNA. Restriction fragments make it possible to locate the positions of restriction sites relative to one another on a DNA molecule. Restriction digestion analysis of DNA with restriction endonuclease enzymes is a very useful and reliable technique to discriminate highly similar parasitic strains within the same species or subspecies. With the discrimination of the strains of parasites using this technique, a way could be laid to get the knowledge of the genetic nature of the parasites which inturn leads to the way of drug design and vaccine development against the parasite. The direct study of nucleotide variation by DNA sequencing or restriction endonuclease analysis can provide an extremely sensitive measure of genetic variation, as long as the DNA sequence analysed is appropriate to the level of variation. Detecting genetic variation and significance of genetic variation within strains is the relative efficiency of electrophoresis and restriction endonuclease analysis of DNA. Mc Manus and Rishi (1989) used three six- base enzymes in their study for *E. granulosus* of British sheep origin. They found an average number of resolvable restriction sites of seven with their rDNA probe and nine with their randomly cloned probe Cuesta – Bandera *et al*., (1988) reported distinct hybridization pattern of restriction endonuclease derived DNA fragments of *E. granulosus* from spain between sheep and donkey strains. The total number of nucleotides surveyed was found to be 288. The analysis of variation in DNA provides a good indicator of genetic differences between inter-breeding groups, such as different species of *Raillietina* or many currently recognized strains of *R. tetragona* which are isolated from different host cycles of varied geographic areas. The experimental studies of restriction digestion of genomic DNA of *R. tetragona* has been made to provide the fundamental steps in the gene cloning process and restriction mapping of the cestode parasite. Restriction mapping is a very

useful and necessary technique for any type of cloning project. Further knowledge of genomic DNA isolation and restriction endonuclease studies help in the identification of species, detection of species variation at the molecular level and in phylogenetic classification of cestode parasites. Assessment of the biological characteristics and other intrinsic criteria of *R. tetragona* are warranted to corroborate the present DNA findings. Further studies including DNA probes, restriction enzymes and restriction mapping of genes will be required to substantiate these observations.



Lane 1: empty

Lane 2: Genomic DNA of *R. tetragona* digested with Bgl I

Lane 3: Genomic DNA of *R. tetragona* digested with Pvu II

Lane 4: Lambda Hind III molecular size marker

Lane 5: Genomic DNA of *R. tetragona* digested with Dra I

Lane 6: Diluted genomic DNA of *R. tetragona*

Lane 7: Stock genomic DNA of *R. tetragona*

Lane 8: Genomic DNA of *R. tetragona* digested with Hinf I

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