# ERIC-PCR ANALYSIS OF *Riemerella anatipestifer* ISOLATES OF KERALA*

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**Abstract**

*Molecular characterisation of six Riemerella anatipestifer isolates were carried out by enterobacterial repetitive intergenic consenses polymerase chain reaction (ERIC-PCR). On analyzing the ERIC-PCR profile, ten to twelve bands per isolate were observed, with a size of approximately 150 bp to 1000 bp. The distance matrices were calculated using the GelQuest - DNA fingerprint analysis software and it was used to construct the phylogenetic relationships among the isolates by the neighbour-joining method. The isolate RA2 was distinct from others. The isolates RA1, RA3, RA4, RA5 having same band pattern approximately at 700 bp were grouped together and separated from RA6. The existence of genetic heterogeneity between the isolates analysed were noticed.*

**Keywords** : *ERIC-PCR, Riemerella anatipestifer, DNA finger printing, new duck disease, Kerala*

Now-a-days, the occurrence of some new or emerging diseases creates great panic among duck owners of the state. New duck disease is the best example of one such disease. *Riemerella anatipestifer* is the causative agent of new duck disease in ducks. The bacterium is a Gram-negative pleomorphic, non-motile and non-sporulating organism. It was isolated from other domestic birds such as geese, turkeys and chicken and was also reported from wild birds and domestic pigs (Sandhu and Rimler, 1997; Hinz *et al*., 1998b). The disease outbreak has been reported from different parts of the state (Priya *et al*., 2008 and Surya, 2011) and from 2008 onwards, several isolates have been identified as *R. anatipestifer*. Studies on identification and characterisation of the isolates has been carried out by Surya (2011) and Shonima (2012).

Acute septicemic form with high mortality among young ducklings under eight weeks of age and chronic form in adult ducks are the often noticed clinical manifestations.

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Because of close similarity, differentiation of *R. anatipestifer* from *P. multocida* is difficult and time consuming by traditional methods at the time of outbreak. To overcome the difficulty, Kiss *et al*. (2007) designed a novel polymerase chain reaction (PCR) based DNA finger printing technology. The method involves the amplification of enclosed genomic DNA between conserved repetitive elements, Enterobacterial repetitive intergenic consensus (ERIC sequences). The location and number of ERIC sequences vary not only between species but also between strains of the same species. As PCR technique was not sensitive to micro environmental factors like growth conditions or available nutrients like traditional biochemical methods, they are more suitable at outbreak situations which require screening of large number of samples. Hence, a study was undertaken to characterize the *R. anatipestifer* isolates by ERIC- PCR.

## Materials and Methods

The genomic DNA of six *R. anatipestifer* isolates (RA1 to RA6) maintained in the Department of Veterinary Microbiology was extracted as per the method of Sambrook and Russell (2001). The ERIC-PCR was carried out as per Kiss *et al.* (2007) using their published primers shown below:

**Forward (F)-**5´ATGTAAGCTCCTGGGGATTCAC 3´
**Reverse(R)-**5´AAGTAAGTGACTGGGGTGAGCG 3´

The composition used in 25 µL single reaction mix for amplification contains EmeraldAmp® GT PCR master mix (12.5 µL), 2.0 µL each of forward primer (10 pM/µL) and reverse primer (10 pM/µL), 2.0 µL of DNA and 6.5 µL of Nuclease free water. The ERIC-PCR programme was carried out in a thermal cycler (Biorad, USA) with an intial denaturation of 95 ºC for 5 min., followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 40 ºC for 3 min. and extension step at 72 ºC for 2 min. with final extension of 72 ºC for 7 min. The amplified PCR products were detected by electrophoresis in one per cent agarose gel at 50V until the bromophenol blue dye migrated more than two-third of the length of the gel. Based on the size, the ERIC-PCR products were separated. By means of GelQuest - DNA fingerprint analysis software and Clustal V software, gel images were compared and analyzed.

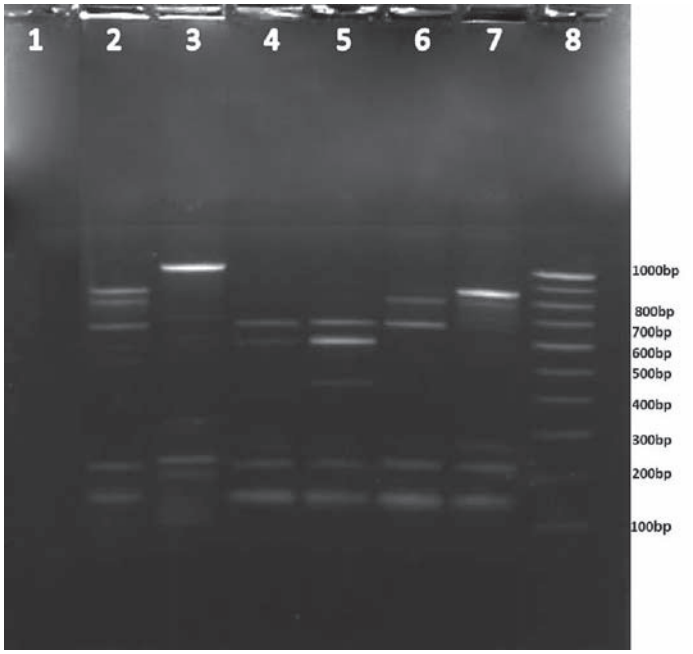
## Results and Discussion

The ERIC-PCR is a molecular biology technique that was first described for the genomes of *Enterobacteriaceae* (Hulton *et al*., 1991). It was suitable for the tentative identification of microorganisms and rapid grouping. Repetitive DNA elements distributed more or less randomly over the genome of eukaryotes and prokaryotes. By doing ERIC-PCR, genetic diversity of the *R. anatipestifer* isolates were studied and the primers (Kiss *et al.*, 2007)) that anneal to the repetitive elements were used. Using agarose gel electrophoresis, the PCR products were separated which generated a species or strain specific pattern.

### *Analysis of ERIC-PCR data*

By analyzing the ERIC-PCR profile (Fig. 1), ten to twelve bands per isolate were observed, with a size of approximately 150 bp to 1000 bp. Different banding pattern were observed for each isolate. One band of approximately 200 bp were common to all isolates. Similar findings were recorded by Pala and Radhakrishnan (2014). One band approximately 150 bp size were similar in RA1, RA3, RA4, RA5 and RA6 except for RA2. A band with a size approximately 300 bp and 1000 bp were found only for RA2. A band with size of approximately 600 bp were found common to RA1, RA3, RA4 and RA6. The RA4 isolate revealed a band with a size of approximately 450 bp. A band of size around 700 bp were common to RA1, RA3, RA4 and RA5. For the identification of different isolates of *R. anatipestifer*, an ERIC-PCR was designed and used by Kiss *et al.* (2007). They also found a band of approximately 700 bp, which was present in ERIC-PCR profiles of all *R. anatipestifer* isolates and opined that the assay helps to differentiate *R. anatipestifer* from *P. multocida* more easily. Bands with size of approximately 300 bp and 1000 bp were found only for RA2. ERIC-PCR profile of RA1, RA5 and RA6 documented similar band size of about 800 bp. One band of approximately 850 bp were found to be common in RA1 and RA6.

**Fig. 1:** ERIC-PCR profile of *R. anatipestifer* isolates




Lane 1: Negative
Lane 2: RA1
Lane 3: RA2
Lane 4: RA3
Lane 5: RA4
Lane 6: RA5
Lane 7: RA6
Lane 8:100 bp DNA ladder

Sixteen isolates of *R. anatipestifer* were subjected to ERIC-PCR analysis by Kardos and Kiss (2005). Kiss *et al*. (2007) studied the genetic relatedness of *R. anatipestifer* strains by ERIC-PCR analysis and noted that the results were reproducible. Hence in each case of repeated experiments, the different strains of *R. anatipestifer* were grouped consistently. On cluster analysis of ERIC-PCR results, Pala and Radhakrishnan (2014) divided the isolates into four variants.

Only distinct and prominent bands were scored for the analysis. Each isolate was scored for the presence or absence (1 or 0) of each band on agarose gel. The distance matrices were calculated using the GelQuest - DNA fingerprint analysis software and the results were directly used to construct the phylogenetic relationships among the isolates by the neighbour-joining method using the same software (Fig. 2). The isolate RA2 was distinct from other isolates. The isolates RA1, RA3, RA4 and RA5 having the same band pattern approximately at 700 bp were grouped together and separated from RA6.

To conclude, this assay helps to differentiate *R. anatipestifer* from *P. multocida* which was difficult and time consuming by traditional methods. The result indicates the existence of genetic heterogeneity between the isolates analysed, and the data is highly useful to design vaccines for the control of the disease.

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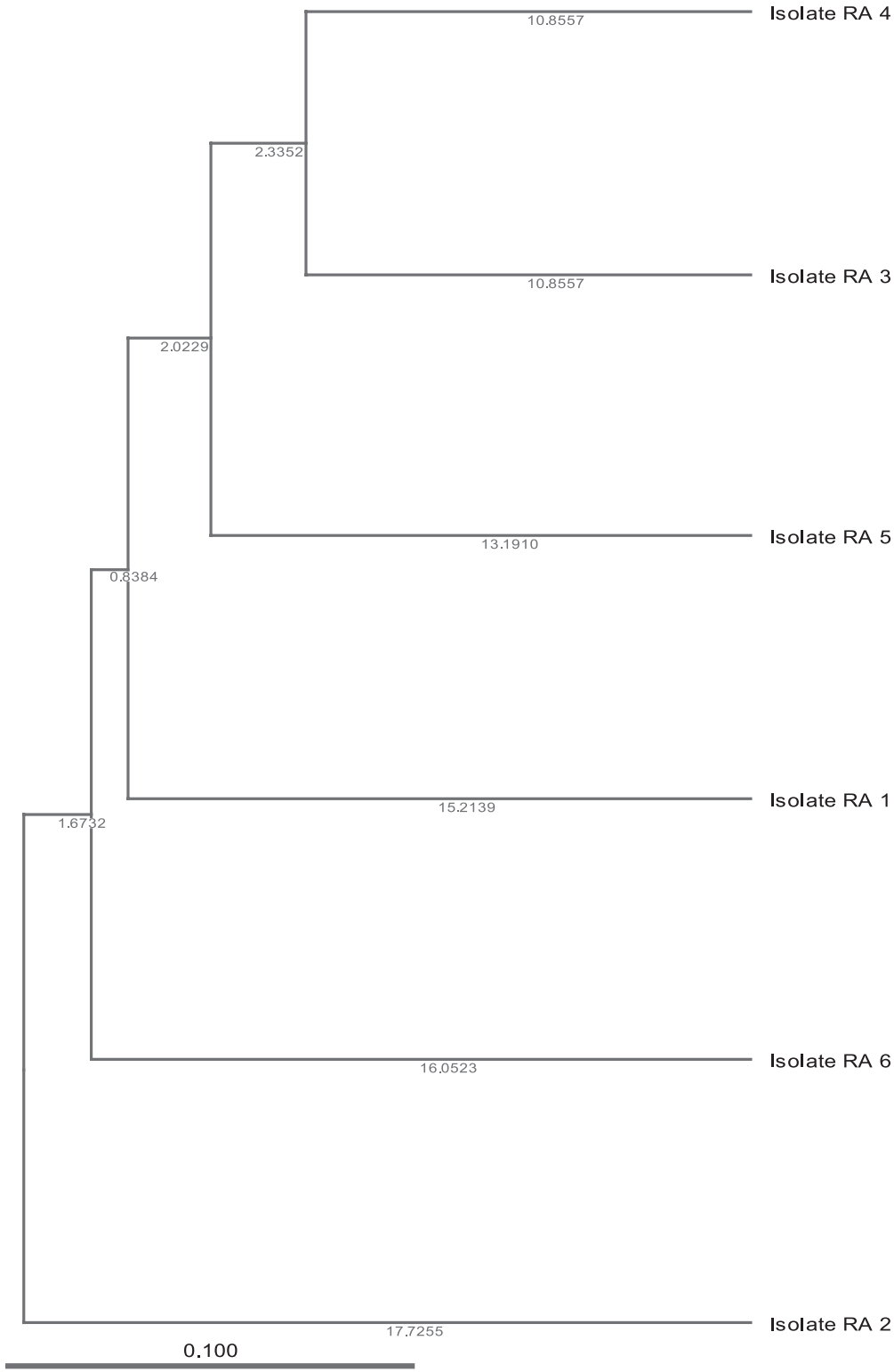
**Fig. 2:** Phylogenetic analysis of ERIC-PCR profile



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