MOLECULAR CHARACTERISATION OF *CHLAMYDOPHILA PSITTACI* ISOLATES BY RESTRICTION ENDONUCLEASE ANALYSIS OF DNA *

Sreeja R. Nair[1], M. Mini[2], V. Jayaprakasan[3] and G. Krishnan Nair[4]
Department of Veterinary Microbiology
College of Veterinary and Animal Sciences
Mannuthy- 680 651, Thrissur, Kerala

Abstract

Reports on the prevalence of abortion due to Chlamydia psittaci (Chlamydophila abortus) in Kerala have indicated a need for the in depth study of the organism. To unearth differences among the isolates of Chlamydia psittaci, at molecular level, restriction enzyme analysis of genomic DNA and plasmid profiling were carried out. Four isolates from four different sources were used in this study. DNA extracted from these isolates were digested by Eco RI, Hae III and Bam HI. Hind III digested Lambda DNA was used as molecular weight marker. The isolates were screened for the presence of plasmids also. Characterisation of chromosomal DNA of Chlamydophila abortus by restriction enzyme analysis revealed a near homogeneity among the isolates. The lack of plasmids in all isolates also indicates the homogeneity of their origin and probably the genetic relationship. Thus, the restriction enzyme digestion analysis with other genetic tools can better resolve the similarity or dissimilarity among the isolates.

Key words: *Chlamydophila abortus, DNA characterisation, restriction endonuclease analysis*

Report on the prevalence of Chlamydiosis in this part of the country has indicated the need for an in depth study on Chlamydial species (Francis, 1988; Sulochana, 1994; Mani, 2001)

Chlamydia psittaci (Chlamydophila abortus) commonly infects a wide variety of mammals and birds and has been implicated in a range of disease conditions (Storz, 1971). In animals, abortion is the most common pathological effect caused by this organism. *Chlamydophila abortus* causing abortion and other forms of disease exist in antigenically distinct forms. But the isolates of *Chlamydia psittaci* from a species or locality cannot be easily differentiated based on the usual diagnostic methods. Hence to understand the epidemiology and epizootology of chlamydiosis in animals and to discern the phylogenic relationship between the species, molecular characterisation techniques are essential.

In this context, the present study was undertaken to compare the genomic DNA of the local isolates of *Chlamydophila abortus* employing restriction endonuclease analysis and to elucidate the presence of extra chromosomal DNA in these isolates.

Materials and Methods

Chlamydial isolates obtained from caprine and bovine abortion which were preserved in the Department of Microbiology, were used in this study (Table 1). The isolates were revived by passaging in six to seven day-old embryonated eggs through yolk sac route. Later the isolates were propagated in McCoy cell line. The elementary bodies (EB) of each isolate prepared from infected cell culture harvest and directly from yolk sac were purified

** Part of M.V.Sc. thesis submitted by the first author to the Kerala Agricultural University, Thrissur*
1. Assistant Professor
2. Professor & Head
3. Associate Dean (Retd.), CVAS, Pookode, Wayanad
4. Professor and Head (Retd.)

by urografin density gradient centrifugation (McClenaghan *et al*., 1984). These purified EB were used for DNA extraction. The homogenised EB in Tris-EDTA sucrose buffer were treated with proteinase k, followed by SDS and then extracted with phenol – chloroform isoamyl alcohol mixture. Nucleic acid was precipitated from the aqueous phase by addition of 1/20 V of 5M NaCl and double volume of ethanol. The concentration and purity of DNA extracted from all the four isolates were assessed by spectrophotometry. Restriction enzyme digestion of the DNA obtained from all the four isolates were done separately with *Eco R 1*, *Hae 111* and *Bam H1*. DNA was digested for overnight in 10 µg amount with 10 x RE assay buffer of 2x concentration and 2U of restriction enzyme per µg of DNA in eppendorf tubes containing distilled water so that the total reaction volume in each tube was 20 µL, under the conditions recommended by the manufacturer. Agarose gel electrophoresis was carried out in horizontal gels with 0.8% agarose containing ethidium bromide (0.5µg/ml) in Tris –borate buffer at 50 V till the loading dye reached three fourth of the anode end of the gel. The molecular sizes of the restriction fragments were estimated by comparison of the distance migrated by them with that of standard molecular weight marker. *Hind 111* digested Lambda DNA was used as molecular weight marker (Fig.1).

Efforts were made to separate and characterise plasmids from all the isolates. Repeated attempts were made to extract plasmid DNA from purified EB by the method of Birnboim and Dolly (1979) except that the lysozyme treatment was omitted. For comparison the plasmids were extracted from *E.coli* V 517 with a similar procedure except that the cell pellet was dissolved in lysozyme at a final concentration of five milligram per milliliter. *E.coli* V 517 was taken as the molecular weight standard having eight plasmids (Fig. 2).

Results and Discussion

In this study, sufficient concentrations of purified EB of *Chlamydia psittaci* were obtained from both Mc Coy cell line and yolk sac membrane. The DNA extracted from the purified EB was subjected to spectrophotometry for assessing the purity. Ratio of OD 260/280 if equal to or more than 1.8 indicated the purity of the DNA samples. It was observed to be more than 1.8 for all the isolates. The concentration of DNA of M- 28, M- 430, M- 121 and P- 156 were 1710 µg/ml, 1130 µg/ml, 1545 µg/ml and 1475 µg/ml respectively (Table 2).

On digestion with *Eco R 1*, the DNA of all the isolates were cleaved into fragments ranging from eight to ten with slight difference in fragment size (Lane 2-5 of Fig. 1). The isolates differed mainly in size of the heavy

Table 1. Details of *Chlamydia psittaci* isolates

Isolate	Source
M-28	Liver of an aborted caprine foetus
M-430	Lung of an aborted caprine foetus
M-121	Liver of an aborted bovine foetus
P-156	Infected yolk sac material obtained from the Department of Microbiology, Veterinary College, Palampur, Himachal Pradesh as reference isolate

Table 2. Concentration and purity of DNA from *C. psittaci* isolates

	M-28	M-430	M-121	P-156
OD 260	0.342	0.226	0.309	0.295
OD 280	0.188	0.122	0.168	0.164
Concentration (µg/ml)	1710	1130	1545	1475
Purity	1.82	1.85	1.84	1.80

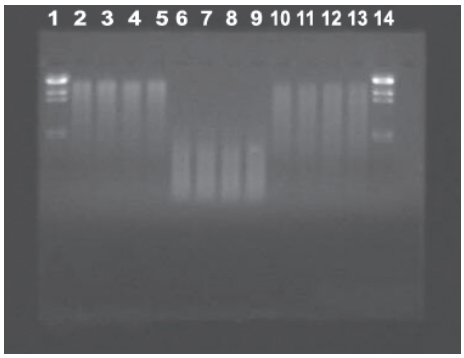


Fig. 1

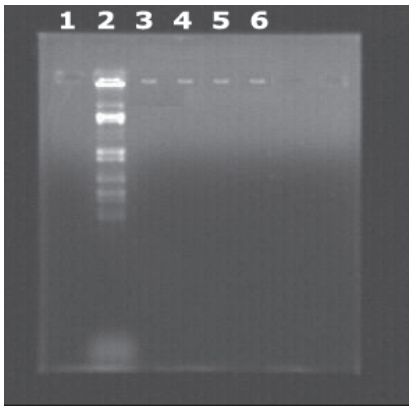
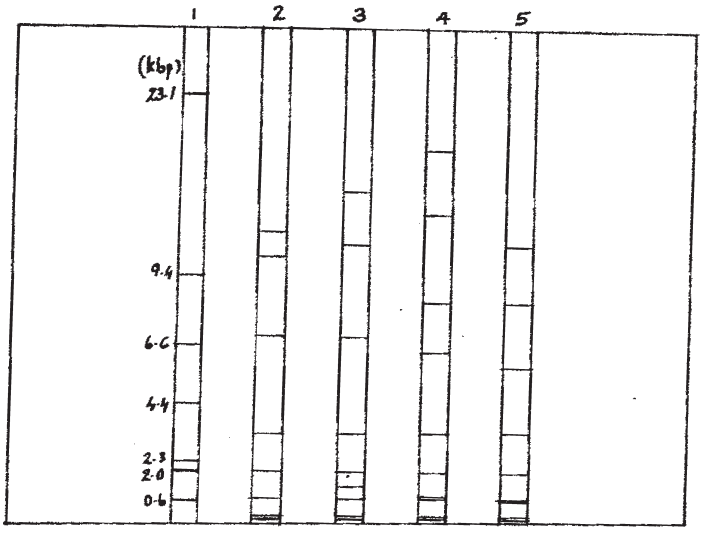


Fig. 2

fragments where as most of the light fragments were similar in size (Table 3). McClenaghan *et al.* (1984) obtained a low number of discrete fragments on *Eco R 1* digestion in higher molecular weight range of 10 to 20 kbp where as the resolution of smaller fragments were poor. They opined that too many fragments cut by the enzymes presented difficulty in their analysis and identification, appearing as poorly resolved bands on electrophoresis.

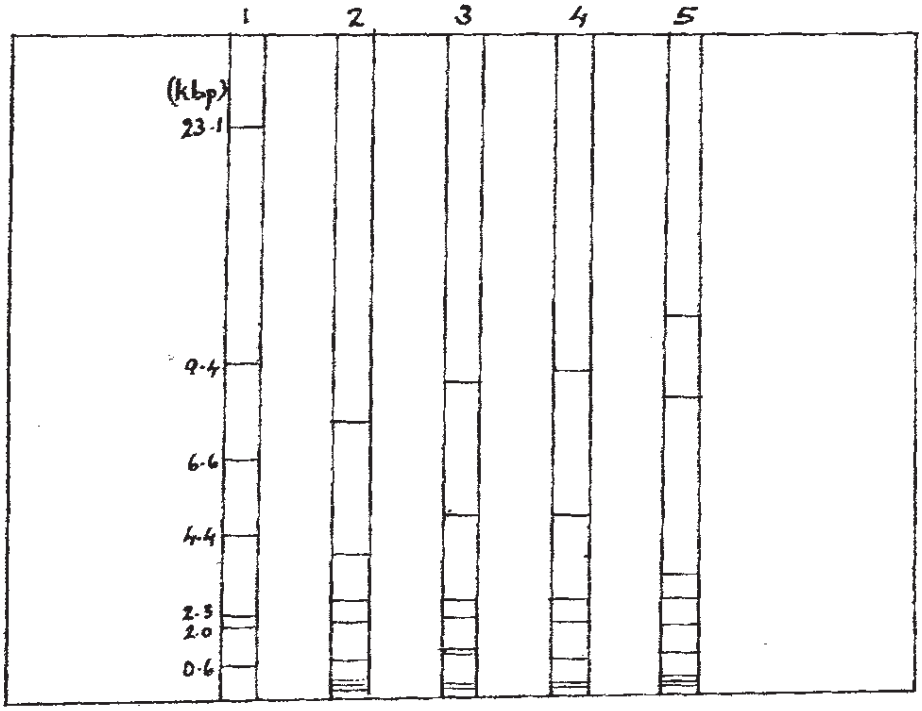
On digestion with *Hae 111*, the number of fragments ranged from seven to nine (Lane 6-9 of Fig. 1). The caprine isolates were having eight fragments each but fragment size varied between the isolates (Table 4). Rodolakis and Souriau (1992) used this enzyme for comparison of various *Chlamydia psittaci* isolates. They observed minor difference between the fragments. Restriction enzymes with four base recognition sequences produced fragments too small for effective resolution in low concentration agarose gels, McClenaghan *et al* (1984). Similar results were observed in the present study.

Table 3. Diagrammatic representation of restriction pattern of *C. psittaci* isolates on digestion with *Eco R1*



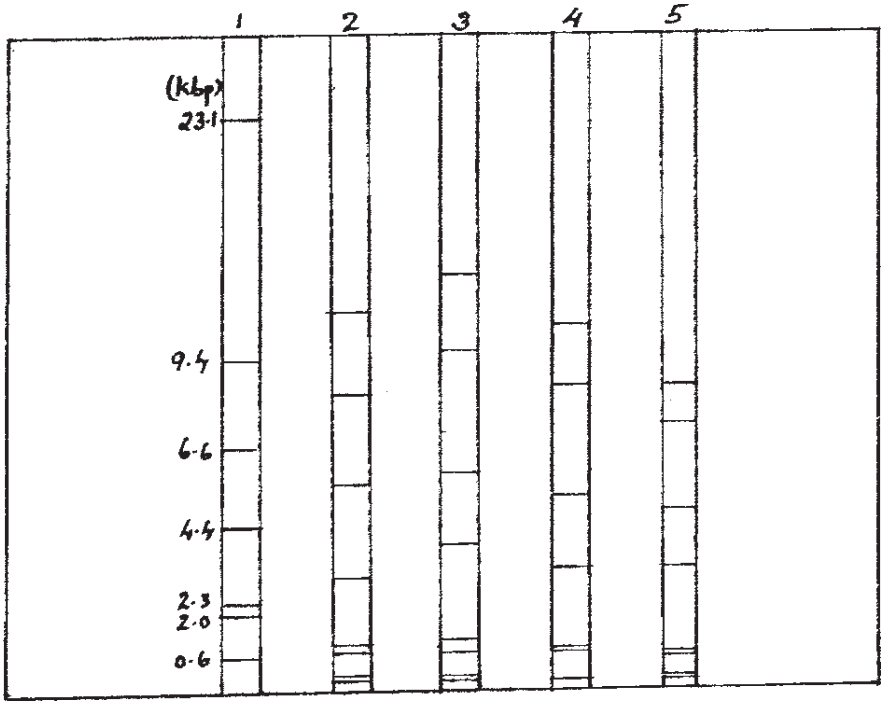
Lane 1 - λ DNA Hind III digest
Lane 2 - M - 28
Lane 3 - M - 430
Lane 4 - M - 121
Lane 5 - P - 156

Table 4. Diagrammatic representation of restriction pattern of *C. psittaci* isolates on digestion with *Hae111*



Lane 1 - λ DNA Hind III digest, Lane 2 - M - 28, Lane 3 - M - 430, Lane 4 - M - 121, Lane 5 - P - 156

Table 5. Diagrammatic representation of restriction pattern of *C. psittaci* isolates on digestion with *Bam H111*



Lane 1 - λ DNA Hind III digest, Lane 2 - M - 28, Lane 3 - M - 430, Lane 4 - M - 121, Lane 5 - P - 156

Digestion of DNA of the isolates with *Bam H 1* yielded eight fragments for all the isolates except M-121 wherein seven fragments were only noticed (Lane 10-13 of Fig 1). But variation was noticed in fragment size among the isolates (Table 5). Rodolakis and Souriau (1992) obtained almost similar fragments with *Bam H 1* and hence recommended its use in the discrimination of strains of *Chlamydophila abortus.*

In this study, efforts were made to separate and characterise plasmids from *Chlamydophila abortus*. Repeated attempts employing the techniques, which had proved fruitful for the isolation of plasmids from gram-negative bacteria including Chlamydia, failed to detect the presence of plasmids in all isolates (Fig. 2). McClenaghan *et al.* (1988) carried out a broad survey to detect plasmids in mammalian strains of *Chlamydia psittaci.* He observed that certain mammalian isolates were free of plasmids. Everett (2000) also reported the absence of plasmids in abortion strains of *Chlamydia psittaci.*

Acknowledgement

The authors are thankful to the ICAR for funding the project on 'Chlamydiosis in livestock with special reference to abortion in livestock'.

References

Everett, K. D. E. 2000. Chlamydia and Chlamydiales more than meets the eye. *Vet. Microbiol.,* **75**: 109-126

Francis, R. 1998. Prevalence of Chlamydial agents in livestock in Kerala. *M.V.Sc. thesis*. Kerala Agricultural University.

Mani, B. K. 2001. Isolation and characterization of *Chlamydia psittaci* with special emphasis on protein profile. *M. V.Sc. thesis*. Kerala Agricultural University.

McClenaghan, M., Herring, A. J. and Aitken, I.D. 1984. Comparison of *Chlamydia psittaci* isolates by DNA restriction endonuclease analysis. *Infect. Immun*., **45** : 384 -389.

McClenaghan, M., HoneyCombe, J.R., Bevan, B.J., Herring, A. J. 1988. Distribution of plasmid sequences in avian and mammalian strains of *Chlamydia psittaci. J. Gen. Microbiol.,* **134**: 559-565

Rodolakis, A.and Souriau, A. 1992. Restriction endonuclease analysis of DNA from ruminant *Chlamydia psittaci* and its relation to mouse virulence. *Vet. Microbiol*., **31**: 263-271.

Storz, J. 1971. Chlamydia induced diseases. In: Thomas C., (Ed.) *Chlamydia and Chlamydia induced diseases.* Springfield, Illinois. pp. 146-246

Sulochana, S. 1994. Personal communication.

❑