Journal of Animal and Veterinary Advances 3 (5): 325-328, 2004

Comparison of Avian Reovirus S1 Genes

[1]Hongzhuan Wu, [2]Narendra K Singh, [2]Robert Locy, [3]Gunn Karyn Scissum and [1]Joseph J. Giambrone
[1]Department of Poultry Science, Auburn University, AL, 36849, USA; [2]Department of Biological Sciences, Auburn University, AL, 36849, USA; [3]Department of Biological Sciences, Alabama State University, AL, USA

Abstract: The S1 genes of a pathogenic avian reovirus (S1133) isolate and a highly attenuated vaccine (SynVac^R) strain, both from the United States (US), were determined and compared. Genes were amplified with a reverse transcription polymerase chain reaction and cloned into a T-vector. Recombinants were subjected to sequencing and sequences analyzed phylogenetically. They were compared with sequences of other avian reoviruses (ARVs) from the US as well as other countries, that were available from the GeneBank database. Reoviruses could be grouped into two major genetypes and five sub-clusters. A close genetic relationship among ARV isolates from different geographic areas was observed. Pathogenic field isolates from all countries belonged to one group, whereas the SynVac^R vaccine strain belonged to a separate group. Results revealed that the genetic diversity in S1 gene could differentiate ARV isolates based on pathogenicity, but not country of origin.

Key words: Comparison, avian, reovirus, S1 genes

Introduction

Avian reoviruses (ARV) are economically important pathogens of commercial poultry. They showed a diverse heterogeneity in pathogenicity. ARVs have been isolated from chickens without clinical disease, as well as from flocks experiencing arthritis, chronic respiratory diseasesand malabsorption syndrome (Fahey and Crawley, 1954; Hieronymus *et al.*, 1983; Kibenege and Wilcox, 1983).

ARVs belong to the genus Orthoreoviruses. They share physical, chemicaland morphological characteristics with mammalian reoviruses (MRV) (Spandidos and Graham, 1976; Schnitzer *et al.*, 1982 and Joklik, 1983). They contain 10 genome segments of double-stranded (ds) RNA, separable into large (L), medium (Mand small (S) classes. The RNA is packaged into a non-enveloped icosahedra double capsid. All ARV-encoded proteins, including 10 structural and 4 non-structural proteins have been characterized (Varela and Benavente, 1994 and Bodelon *et al.*, 2001). Sigma C protein, encoded by ARV S1 gene (Varela and Benavente, 1994 and Shapouri *et al.*, 1995), is 326 amino acids in length. Studies with reassortant ARVs showed that the S1 gene was involved in both viral replication and pathogenesis. It possessed both type and broad specific eptiopes and appeared to be analogous to the MRV sigma 1 protein (Wickramasinghe *et al.*, 1993 and Shwapouri *et al.*, 1996).

Differences in genomic coding sequences for individual proteins exist between ARV and MRV. Despite being an important poultry pathogen, little molecular data is available on ARV. Objectives of this study were to present sequence information for ARV S1 gene and examine sequence diversity among pathogenic isolates from several countries and a vaccine strain from the United States (US).

Materials and Methods

Virus Propagation and RNA Preparation: Two ARVs (S1133 and SynVac^R) were used. The S1133 strain is a standard challenge strain. SynVac^R is a cell cultural vaccine virus (Lohman Animal Health, Gainesville, Ga). It is an attenuated, cell culture propagated S1133 strain. ARVs were propagated in nine-day-old embroynating SPF eggs by the chorioallantoic membrane (CAM) route. Allantoic fluid was collected for RNA extraction after embryo death. Viral RNA was extracted from 500 μl allantoic fluid using 500 μl TRIZOL reagent (Life Technologies INC.,) following the manufacturer's protocol.

Reverse Transcription Polymerase Chain Reaction (RT-PCR): To amplify the full-length sigma C encoding gene of S1133 and SynVac^R, total viral RNA was used to generate cDNA clones by reverse transcription and polymerase chain reaction (RT-PCR). cDNA synthesis was according to the Superscript™ Preamplification System for First Strand cDNA Synthesis (Life Technologies, GIBCO BRL). 1μg of total RNA was usedand reverse transcription was carried out at 42°C for 1hr. PCR primers, used to amplify entire or a portion of sigma C encoding gene, are shown in table 1. PCR reactions were performed in 50 μl containing 10X PCR buffer, 2.5mM manganese chloride solution, 300μM dNTPs, Taq DNA polymerase (5units)and 5μM of the primer pairs (Reo-1/Reo-2 or Reo-3/Reo-4)and 1μl cDNA. PCR reactions were subjected to 30 cycles consisting of denaturation for 1 minute at 94°C, annealing for 1 minute at 50°C and extension for 1minute at 72°C, and one final extension cycle at 72°C for 10 minutes.

After completion of PCR, 10μl of the reaction mixture was loaded onto a 1% agarose gel containing 5μg/ml ethidium bromide for electrophoresis and subsequent visualization by UV transillumination.

Cloning and Sequencing of S1 Gene: To confirm that amplified PCR products contained the expected S1 gene fragments, resulting products were purified and cloned into a T-vector according to the manufacturer's protocol (Promega Co). Each purified PCR product (100ng) of 980 or 810bp cDNA fragment length was cloned into the T-vector with a 1h ligation at room temperature using T4 DNA ligase. Recombinant plasmids transformed E.coli. DNA minipreps were performed on white putative colonies (blue/white selection)and were characterized with EcoR I digestion to determine if they were the correct inserts. Clones with the correct sizes were purified with a Qiagen kit and used in sequencing reactions using an automatic laser fluorescence DNA sequencer.

Phylogenetic Tree Analysis of Avian Reovirus S1 Gene: Nucleotide sequences of seven US isolates (S1133, SynVac^R , 1733, 176,138 and 2408), two from Taiwan (601S1 and 918)and two Australian isolates (Ram1 and Som4) were aligned with the DNA star software package. (DNAstar Co.). The phylogenetic relationship of ARVs were analyzed with this package. All USA isolates belong to a single serotype. The serotypes of the foreign viruses are not known.

Results

Propagation of Avian Reoviruses in SPF Chicken Embryos: Both ARVs caused curling and stunting in embryos and formed plaques on CAM.

Isolation of Avian Reovirus S1 Gene with a RT-PCR: Correct size fragments (980 bp and 810bp) of the S1 gene were obtained after RT-PCR with primers Reo-1/Reo-2 and Reo-3/Reo-4. There were no deletions or insertions in the S1 gene of these strains. (Fig. 1).

Characterization of the cDNA Clone with Restriction Enzyme Digestion: Identification of cDNA clones was confirmed by restriction enzyme digestion analysis (Fig. 2) and sequencing with an Automatic Laser Fluorescene DNA sequencer. Nucleotide sequence data of SynVac^R strain was deposited in GenBank database and assigned accession number AY149381 (Table 2).

Phylogenetic Tree Analysis of Avian Reovirus S1 Gene: A phylogentic tree was provided in Fig. 3. Sequences of the two ARVs were aligned with 8 isolates from the US, Taiwanand Australia, respectively. Two major genotypes were evident. Group I contained viruses from US (S1133, 1733, 2408, 176 and 138), Taiwan (601SI and 918) and Australia (Ram-1 and Som4), whereas group II included only SynVac^R. Results indicated that the S1 gene exhibited little variation. A close genetic relationship was observed among ARV isolates from different geographic areas.

Table 1: Primers used in this study

Primer	Sequences (5′—3′)	Target position	Expected size (bp)
Reo-1	ATGGCGGGTCTCAATCCATCG	S1 1-20	980
Reo-2	TTAGGTGTCGATGCCGGTACG	S1 981-961	
Reo-3	CGAAGAGAGAGGTCGTCAGCTT	S1 25-45	810
Reo-4	ATACGTCCACAGGGAACGAC	S1 835-815	

Table 2: Avian reovirus strains used in the phylogenetic analysis

Name	origin	Gene Bank accession number
S1133	US	AF330703.1
SynVac^R	US	AY149381
1733	US	AF004857
176	US	AF218358
138	US	AF218359
2408	US	AF204945
601SI	Taiwan	AF240947
918	Taiwan	AF297215
Ram-1	Australia	gi601926
Som4	Australia	gi294004

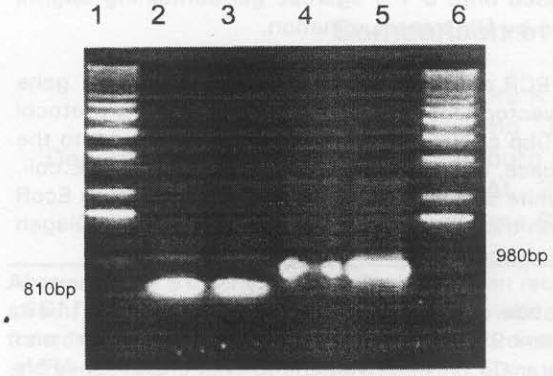

Fig. 1: Isolation of two avian reovirus S1 gene with a RT-PCR
Lane 1 ,6 1kb DNA ladder
Lane 2 S1133 cDNA with primer Reo-1 and Reo-2
Lane 3 SynVac^{R} strain cDNA with primer Reo-1 and Reo-2
Lane 4 S1133 cDNA with primer Reo-3 and Reo-4
Lane 5 SynVac^{R} strain cDNA with primer Reo-3 and Reo-4

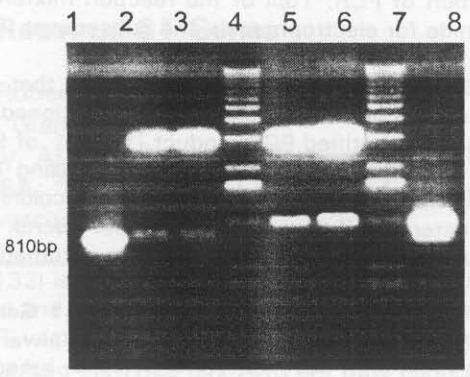

Fig. 2:Characterization of the cDNA clone with restriction enzyme digestion
Lane 1. 810bp PCR product
Lane 2. S1133 clone-1 digested with EcoR I
Lane 3. SynVac^{R} clone-2 digested with EcoR I
Lane 4, 7 1kb DNA ladder
Lane 5 S1133 clone-3 digested with EcoR I
Lane 6 SynVac^{R} clone-4 digested with EcoR I
Lane 8 980bp PCR product

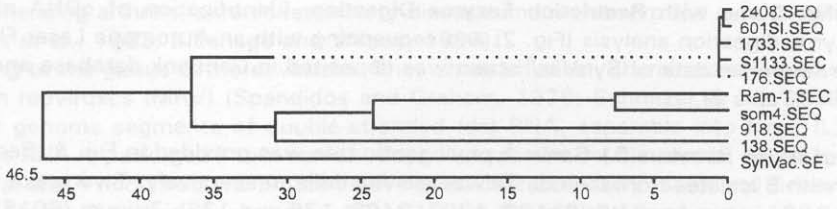

Fig.3: Dendrogram of the phylogenetic tree of avian reovirus isolates, which was inferred from the S1 gene by DNA star. Length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events. A dotted line on a phonogram indicates a negative branch length, a common product of averaging.

Discussion

The sigma C protein is the minor-capsid protein and is encoded by the largest open reading frame (ORF) of the S1 segment. Studies showed that S1 segment was involved in both viral replication and pathogenesis (Robertson and Wilcox, 1983; Ni and Raming, 1993 and Theophilos *et al.,* 1995). Although only a limited number of sequences of S1 gene have been reported, it indicated the presence of type-specific sequences in S1 gene. This gene has evolved quickly, resulting in a wide heterogeneity in pathogenicity and neutralizing antigens (Clark *et al.,* 1990; Robertson and Wilcox, 1986; Rosenberger *et al.*, 1989 and Sterner *et al.,* 1989). ARV strains exhibiting the unusual subgroup or diversity in electrophoresis patterns were also demonstrated. These have lead to at least eleven serotypes and (Takase *et al.*, 1987 and Lee *et al.*, 1992). Although all field isolates isolated from the US and vaccines developed in the US belong to the same serotype, subtypes have been established based on serologic testing (Giambrone and Solano, 1988). Genotyping offered a useful tool to differentiate avian reovirus more accurately and rapidly (Lin *et al.*, 1991 and Heppell *et al.,* 1992).

Data herein showed there was a relationship between the S1 gene sequence and pathotype. All field isolates, regardless of the country of origin, could be separated from the vaccine strain. Differentiation of vaccine from pathogenic field strains is important in the diagnosis and epidemiology of the disease. Phylogenetic tree analysis can facilitate the setup of this database. Genetic divergence made it possible to differentiate ARV isolates. When designing a vaccination program for ARV, it is important to identify the serotype or genotype of the field isolates as cross protection may be limited among isolates. Our information can be used for improved detection and control of ARVs. Future studies will examine more S1 genes of avian reoviruses from both vaccine and field strains to draw more detailed conclusions of the classification of avian reoviruses at the nucleic acid or amino acid levels.

Acknowledgements

This project was supported by a USDA-IFAFS grant(#00-52100-9705).We are grateful to Zhuyan Guo for technical help.

References

Bodelon, B., L. Labrada, J. Martinez-Costas and J. Benavente, 2001. The avian reovirus genome segment S1 is a functionally tricistronic gene that expresses one structural and two nonstructural proteins in infected cells. Virology, 290:181-191.

Clark, F.D., Y. NI, E.W. Collisson and M.C. Kemp, 1990. Characterization of avian reovirus strain-specific polymorphism. Avian Diseases, 34:304-314.

Fahey, J.E. and J.F. Crawley, 1954. Studies on chronic respiratory disease of chickens II. isolation of a virus. Canadian J of Comparative Medi., 18:13-21.

Giambrone, J.J. and W. Solano, 1988. Serological comparison of avian reovirus isolates using virus neutralization and an enzyme-linked immunosorbent assay. Avian Dis., 32:678-680.

Heppell, J., L. Berthiaume, E. Trarrab, J. Lecomte and M. Arella, 1992. Evidence of genomic variations between infectious pancreatic necrosis virus strains determined by restriction fragment profiles. J. of General Virology, 73:2863-2870.

Hieronymus, D.R.K., P.Villegas and S.H. Kleven, 1983. Identification and serological differentiation of several reovirus strains isolated from chickens with suspected malabsorption syndrome. Avian Dis. 27:246-254.

Joklik, W.K. (Ed), 1983. The Reoviridae. Plenum, New York.

Kibenege, F.S.B. and G.E. Wilcox, 1983. Tenosynovitis in chickens. Veterinary Bulletin, 53:431-443.

Lee, L.H., Y.H. Wang and J.H. Shien, 1992. Serological characterization of reoviruses isolates from avian species in Taiwan. Journal of the Chinese Society of Vet. Sci., 18:69-72.

Lin, Z., A. Kato and S. Ueda, 1991. A new typing method for the avian infectious bronchitis virus using polymerase chain reaction and restriction enzyme fragment length polymorphism. Archives of Virology, 116:19-31.

NI, Y. and R.F. Ramig, 1993. Characterization of avian reovirus-induced cell fusion: the role of viral structural proteins. Virology, 194:705-714.

Robertson, M.D. and G.E. Wilcox, 1986. Avian reovirus. Veterinary Bulletin, 56:174.

Rosenberger, J.K., F.J. Sterner, K.P. Botts, K.P. Lee and A. Margolin, 1989. *In vitro* and *in vivo* characterization of avian reoviruses. I. Pathogenicity and antigenic relatedness of several avian reovirus isolates. Avian Dis., 33:35-544.

Schnitzer, T.K., T. Ramos and V. Gouvea, 1982. Avian reovirus polypeptides: analysis of intracellular virus-specified products of virion, top componentand cores. J.Virol., 43:1006-1014.

Shapouri, M.R.S., M. Kane, M. Letarte, J. Bergeron, M.Arella and A. Silim, 1995. Cloning, sequencing and expression of the S1 gene of avian reovirus. J. Gen Vir., 76:1515-1520.

Shapouri, M.R.S., D.Frentte, R. Larochelle, M. Arella and A.Silm, 1996. Characterization of monoclonal antibodies against avian reovirus strain S1133. Avian Path., 25:57-67.

Spandidos, D.A. and A.F. Graham, 1976. Physical and chemical characterization of an avian reovirus. J. Vir., 19:968-976.

Sterner, F.J., J.K. Rosenberger, A.Margolin and M.D. Ruff, 1989. In *vitro* and *in vivo* characterization of avian reovirus. II. Clinical evaluation of Chickens infected with two avian reovirus pathotypes. Avian Dis., 33:545-554.

Takase, K., F. Nonaka, M. F. Yamamoto and S.Yamada, 1987. Serological and pathogenic studies on avian reoviruses isolated in Japan. Avian Dis, 31:464-469.

Theophilos, M.B., J.A. Huang and I.H. Homes, 1995. Avian reovirus ó6C protein contains a putative fusion sequence and induces fusion when expressed in mammalian cells. Virology, 208:678-684.

Varela, R. and J. Benavente, 1994. Protein coding assignment of avian reovirus S1133. J. of Vir., 68:6775-6777.

Wickramasinghe, R., J.Menager, C.E. Enriques and G.E. Wilcox, 1993. Avian reovirus proteins associated with neutralization of virus infectivity. Virology, 194:688-696.