RESTRICTION FRAGMENT LENGTH POLYMORPHISMS REVEAL A NEW MOLECULAR GROUP OF INFECTIOUS BURSAL DISEASE VIRUS IN SAUDI ARABIA.

By

A. N. Alkhalaf

College of Agriculture and Veterinary Medicine, Qussim University, Al-Qussim-Buridah 81999, P.O. Box 1482

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Abstract: *Infectious bursal disease virus (IBDV) causes immunosuppression in chickens and high condemnations in slaughter plants. A total of 87 samples were obtained from fifteen commercial chicken flocks and nine backyard chicken flocks that have different ages and vaccination programs. The reverse transcriptase-polymerase chain reaction (RT-PCR) technique was used to detect the viruses and Restriction Fragment Length Polymorphisms (RFLP) was used to compare the RT-PCR products among the viruses that were detected. The restriction enzymes that were used in the RFLP are BstNI and MboI. All backyard flock samples were negative to IBDV. Four samples from commercial flocks were positive for IBDV using the RT-PCR and the RFLP was performed on the RT-PCR product of these samples. Two samples had RFLP bands that were different from any other known RFLP patterns and the other samples had RFLP bands that matched classical vaccine strain patterns. In conclusion, a new molecular group of IBDV may be present in Saudi Arabia that is different from any existing molecular group.*

Abbreviations: IBDV = Infectious bursal disease virus; RT-PCR = reverse transcriptase-polymerase chain reaction; RFLP = Fragment Length Polymorphisms, DMSO = dimethyl sulfoxide solution, hv = hyper-variable region, AC-ELISA = Antigen capture enzyme linked immunosorbent assay

INTRODUCTION

Infectious bursal disease virus (IBDV) is the etiologic agent of an infectious, contagious, and immunosuppressive disease of young chickens. The virus is ubiquitous in the environment of modern commercial poultry and is considered to be among the most economically important infectious diseases affecting the commercial industry (5). Two serotypes of the virus have been recognized and designated as serotypes 1 and 2 (1,6). All known serotype 2 strains are infectious to chickens but apathogenic (5). Serotype 1 viruses are associated with clinical disease in young chickens and are comprised of a number of strains or subtypes that can differ in their antigenic, pathogenic, and genetic profiles (4).

Many strains of the virus have been sequenced or cloned. This information has been useful in identifying areas of variability between different virus strains (5,7). One of the most investigated areas of the IBDV genome is an extremely heterogeneous area referred to as the hyper-variable (hv) region which codes for the immunodominant viral epitope (1). The genetic heterogeneity of this area has been demonstrated by reverse transcription polymerase chain reaction-restriction fragment length polymorphisms (RT/PCR-RFLP) of international field isolates of IBDV (3).

Variant strains of IBDV which circumvent classical vaccinal induced immunity were detected in the USA (4). Molecular techniques and primers that are directed to the (hv) region of the VP2 gene of IBDV were used to diagnose and identify molecular differences in the VP2 gene of IBDV from chicken samples (2, 3). In this work, RT/PCR-RFLP techniques were used to detect and differentiate IBDV isolates from chicken bursal tissues.

MATERIALS AND METHODS

Samples collections: Samples were collected from twenty-four flocks (fifteen commercial chicken flocks and nine backyard chicken flocks). A total of 87 samples were collected. Four samples each containing five bursas were collected from each commercial flock and three samples each containing two bursas were collected from each backyard flock and placed in –80°C freezer until used.

Preparation of viral RNA: preparation of the viral RNA for RT-PCR was conducted as previously described (2,3). Briefly, bursal tissue samples were rinsed in TNE buffer (10 mM Tris-HCl , 100 mM NaCl, 1 mM ethylenediaminetetraacetic acid). The tissues were homogenized in TNE buffer and the supernatant was extracted with an equal volume of chloroform. Sodium dodecyl sulfate (Sigma Chemical Co.-St. Louis, MO)

and proteinase-K (Sigma) were added to a final concentration of 0.5% and 1.0 mg/ml, respectively. The mixture was incubated at 37^0 C for 1 hour and then extracted with equal volumes of acid phenol, pH 4.3 (AMRESCO, Solon, OH), followed by chloroform: isoamyl alcohol (24: 1). Total RNA was precipitated from the aqueous phase by ethanol. Precipitated RNA was pelleted by centrifugation and resuspended in a 100 µl volume of 90% dimethyl sulfoxide solution (DMSO).

RT-PCR / RFLP: The procedure used for RT-PCR was previously described (2,3). Extracted viral RNA was reverse transcribed and PCR amplified using the GeneAmp RNA PCR kit (Perkin Elmer, Roche Molecular Systems Inc., Branchburg, NJ) according to the manufacturer's protocols. The RFLP was conducted on the RT-PCR products using the *Bst*NI and *Mbo*I restriction enzymes as described (2,3). The RT/PCR products were electrophoresed in 1.0% agarose gels (SeaKem®-LE, FMC Bioproducts, Rockland, ME) which was prepared with 1X-TBE buffer and visualized using ethidium bromide (EtBr) staining and uv-illumination.

RESULTS AND DISCUSSION

There were no positive samples from the twenty-seven samples from backyard. Four viruses were detected from commercial flocks using the RT-PCR. The RT-PCR products were subjected to restriction enzymes (the *Bst*NI and *Mbo*I) using the RFLP technique. Two viruses that had the same band patterns that resembled a classical vaccine are shown in fig 1 (line 2 and 4). The other two viruses had bands that looked alike and are deterrent from any molecular group that have been reported earlier (2,3) as shown in fig 1 (line 1 and 3).

The viruses that have RFLP patterns like the classical vaccine came from bursal tissues from twenty one day-old chicks, which were vaccinated in the drinking water a week before sampling. The viruses that have the new RFLP patterns came from bursal tissues from thirty five day-old chicks. Because these samples came from the field, contamination with more than one virus is possible which gives bands that are hard to predict. These viruses that were detected in RT-PCR/RFLP could be different vaccine viruses infecting one bird.

New viruses are detected in different places around the world using molecular technique or serology (3). These data suggest that viruses continue to change and may circumvent the immune system. .

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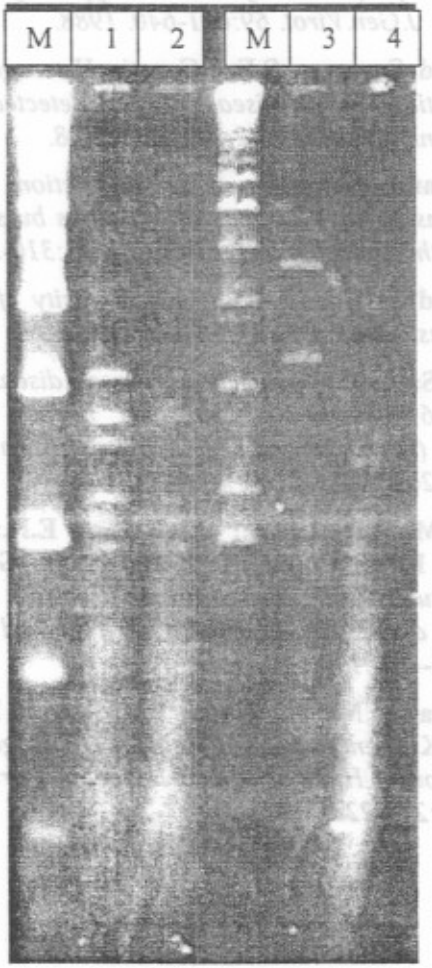
Table 1. Number of flocks and samples and the results of the RT-PCR and RFLP

Flocks #	Samples #	# Positive Samples for RT-PCR	# Positive Samples for RFLP
15 commercial flocks	60	4	4
9 backyard flocks	27	0	0

Fig. 1. RFLP results of IBDV strains detected in commercial flocks.



M = marker, 1 = cut with *Bst*NI (new virus), 2 = cut with *Bst*NI (classical virus), 3 = cut with *Mbo*I (new virus), 4 = cut with *Mbo*I (classical virus)

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