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Molecular characterization of field isolates and vaccine strains of infectious bursal disease virus

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Abstract

The present investigation was conducted to study the genetic heterogenicity and molecular polymorphism among the field isolates and vaccine strains of infectious bursal disease virus (IBDV). Samples of bursa of Fabricius from 15 suspected outbreaks of infectious bursal disease (IBD) were subjected to agar gel precipitation test (AGPT), virus isolation and reverse transcription-polymerase chain reaction (RT-PCR) combined with restriction fragment length polymorphism (RFLP). Nine out of 15 samples were found positive in AGPT while 14 were found positive both by virus isolation and RT-PCR. PCR amplified 474 bp fragment from the variable region of VP2. *Sac* I, *Stu* I, *Alu* I, *Ssp* I and *Mbo* I restriction enzymes were used for characterization of all the 14 IBDV isolates and four reference vaccine strains. *Sac* I, *Stu* I, *Alu* I and *Ssp* I could differentiate classical virulent IBD (cvIBD) vaccine virus strains from very virulent IBD (vvIBD) field isolates by their varying restriction patterns. Based on above results two field isolates (VPL and VMK) were placed in cvIBD virus group and 12 field isolates were placed in vvIBD virus group. Virus neutralisation test (VNT) using rabbit raised Georgia strain anti-serum, however, could not differentiate between cvIBD virus and vvIBD virus. It was concluded that RT-PCR combined with RFLP assay using restriction enzymes *Sac* I,

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Stu I, *Alu* I and *Ssp* I can be used for rapid differentiation and classification of field isolates of IBDV.

Keywords: Field isolates; Infectious bursal disease virus; Restriction fragment length polymorphism; Reverse transcription-polymerase chain reaction; Vaccine strains; Virus neutralization test

Résumé

La présente étude a été conduite pour étudier l'hétérogénéité génétique et le polymorphisme moléculaire des souches de virus isolées dans la nature et les souches vaccinales de la bursite infectieuse.

Les échantillons de bourse de Fabricius provenant de quinze cas présumés de bursite infectieuse ont été soumis au test de précipitation sur gel. L'isolement du virus a été recherché de même que RT-PCR combinée avec la RFLP.

Neuf des quinze échantillons se sont montrés positifs au test de précipitation sur gel alors que quatorze ont été trouvés positifs par isolement du virus, RT-PCR.

La PCR a amplifié 474 fragments BP de la région variable de VP2. Des enzymes de restriction ont été utilisés pour la caractérisation des quatorze échantillons isolés et des quatre souches de vaccin de référence. Deux isolats de virus sauvage ont été classés parmi les souches virulentes classiques et douze parmi les souches très virulentes. Il est suggéré que le test RT-PCR combiné avec le RFLP utilisant les enzymes de restriction *Sac1, Stu1, Alu1, Sps1* peuvent être utilisés pour une différenciation rapide et une classification des virus sauvages de bursite infectieuse.

Mots cles: Virus de la bursite infectieuse; Virus sauvage; RT-PCR; RFLP; Souche vaccinale

1. Introduction

Infectious bursal disease (IBD) is an acute, highly contagious, viral infection of poultry causing heavy economic losses worldwide. Infectious bursal disease virus (IBDV) affects 3–6 week-old young chicks, has predilection for bursa of Fabricius and causes prolonged immunosuppression leading to concurrent viral and bacterial infections along with vaccination failures [1]. The capsid of IBDV contains five structural proteins, i.e. VP1, VP2, VP3, VP4 and VP5 [2]. Serotype-specific antigenic determinants inducing neutralizing antibodies are located on VP2, whereas group-specific monoclonal antibodies recognize antigenic sites located on structural proteins, VP2 and VP3 [3]. Two serotypes of IBD virus can be differentiated by virus neutralization test (VNT). Serotype-1 contain strains that are pathogenic to chickens, whereas strains of serotype-2 do not cause disease in chickens [4]. Serotype-1 strains can be broadly grouped into classical virulent strains and very virulent strains [5]. The differentiation between very virulent IBDV and classical virulent IBDV is not possible with serological techniques like agar gel precipitation test (AGPT), VNT, ELISA, etc. Moreover, molecular differentiation of IBDV strains