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EXPERIMENTALLY INDUCED DISEASE

Comparative Pathogenicity of Malaysian QX-like and Variant Infectious Bronchitis Virus Strains in Chickens at Different Age of Exposure to the Viruses

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Summary

Infectious bronchitis viruses (IBVs) circulating in Malaysia are classified into two groups as Malaysian QX-like and variant strains. In this study, the pathogenicity of IBS130/2015 (QX-like) and IBS037A/2014 (variant) IBVs in 1-day-old and 30-day-old specific pathogen free (SPF) chickens was characterized. Both strains caused respiratory and kidney infections based on immunohistochemistry (IHC), real-time quantitative polymerase chain reaction (qPCR) and a ciliostasis study; however, the results showed that the QX-like strain was more pathogenic, caused higher mortality and showed higher tissue tropism for the kidney than the variant strain. In contrast, despite causing low or no mortality depending on the age of the infected chickens, the Malaysian variant strain showed high tissue tropism for the respiratory tract compared with the QX-like strain. IHC and qPCR indicated the presence of both IBV strains in the epithelial lining of villi in the jejunum and the caecal tonsil; however, no pathological changes were detected in these organs. Both the Malaysian QX-like and variant IBV strains are able to infect the respiratory tract and kidney of chickens irrespective of age.

Keywords: chicken; infectious bronchitis virus; Malaysian QX-like and variant strains; pathogenicity

Introduction

Avian infectious bronchitis virus (IBV) is the causative agent of an economically important disease, commonly known as infectious bronchitis (IB), affecting poultry industries all over the world (Jackwood, 2012). Although, IBV is a respiratory virus, it can infect other epithelial cells namely those in the kidney, oviducts and the gastrointestinal tract (Raj and Jones, 1997; Villarreal *et al.*, 2007; Jackwood and de Wit, 2013). The severity of disease and tissue tropisms depends predominantly on the strains of IBV and age of chickens at infection. In some cases, disease severity also depends on secondary infection (Avellaneda *et al.*, 1994), routes of inoculation (Uenaka *et al.*, 1998) and environmental conditions (Raj and Jones, 1997).

Chickens of all ages are susceptible to IBV infection; however, the incidence of IB seems higher in those <6 weeks of age. In addition, the virulence of the strains of IBV depends on the age of infection. In particular, chickens <2 weeks old typically show more severe nephritis and higher mortality than older chickens (Jackwood and de Wit, 2013) and the pathogenicity of Massachusetts serotypes decreases when chickens are inoculated at an older age (Crinion

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and Hofstad, 1972). However, another study showed an inverse of age resistance with the H52 strain after intravenous inoculation (Macdonald *et al.*, 1980; Jackwood and de Wit, 2013).

The QX serotype was first isolated almost two decades ago following an outbreak of the disease in poultry in China (Yu *et al.*, 2001). The QX-like strains have become the most widely distributed strains and have been reported in many European countries (Domanska-Blicharz *et al.*, 2006; Worthington *et al.*, 2008; Abro *et al.*, 2012), the African continent (Ducatez *et al.*, 2009; Abolnik, 2015) and in the Asian countries (Mase *et al.*, 2004; Lee *et al.*, 2008; Pohuang *et al.*, 2011). In early reports, IB caused by the QX strain was characterized predominantly by the presence of a swollen proventriculus (Wang *et al.*, 1998). Subsequent studies also showed lesions in the trachea (Pohuang and Sasipreeyajan, 2013), kidney (Landman and Dwars, 2005; Gough *et al.*, 2008; Pohuang and Sasipreeyajan, 2013) and oviduct (Landman and Dwars, 2005) and reduction of egg production (Yu *et al.*, 2001; Liu and Kong, 2004; Landman and Dwars, 2005).

In addition to IBV QX-like strains, other IBV variants including T (Australia), Holte and Gray (USA), B1648 (Belgium) and AZ2374 (Italy) are known as nephropathogenic strains that cause histopathological lesions in the trachea and kidneys, as well as mortality (Ignjatovic *et al.*, 2002). The molecular epidemiology of IBV in Malaysia has not been widely investigated. Recently, we reported the occurrence of both the Malaysian QX-like and variant strains in commercial poultry farms in Malaysia (Khanh *et al.*, 2017); however, the pathogenicity of these two IBV strains has not been studied by experimental infection of specific pathogen free (SPF) chickens. The aim of this study was to characterize the pathogenicity of Malaysian QX-like and variant strains of IBV by inoculation of the viruses into 1- and 30-day-old SPF chickens.

Materials and Methods

Ethical Approval

The study was approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), with reference number UPM/IACUC/AUP-R043/2016.

Virus Strains

IBV isolates IBS130/2015 (QX-like strain) and IBS037A/2014 (Malaysian variant strain) were initially isolated from IB outbreaks in 2015 and 2014, respectively. Virus isolation, propagation and molecular characterization of these two isolates has been described (Khanh *et al.*, 2017). The IBS130/2015 isolate at passage 5 and the IBS037A/2014 isolate at passage 6, which cause specific lesions of IBV infection in chicken embryos (i.e. embryonic mortality, stunting and curling with feather dystrophy), were used to inoculate the SPF chickens.

Chickens

Eight-day-old embryonated SPF eggs (VALO Biomedia, Adel, Iowa, USA) were incubated at 37°C in a sterile hatchery facility for 13 days in the Laboratory of Vaccines and Immunotherapeutics, Institute of Bioscience, UPM. After hatching, 1-day-old SPF chickens were reared in stainless steel cages in the experimental animal facility of the Faculty of Veterinary Medicine, UPM. Chickens were supplied with feed and water *ad libitum*.

Experimental Design

A total of 240 SPF chickens were divided into four virus-infected groups (groups 1, 2, 3 and 4; $n = 46$ chickens per group) and two control uninoculated groups (groups 5 and 6; $n = 28$ chickens per group). Chickens in groups 1 and 2 were inoculated via the intraocular route with 0.05 ml of medium containing 1×10^5 median embryo infectious dose $(EID)_{50}$ of IBS130/2015 at the ages of 1- and 30-days old, respectively. Meanwhile, chickens in groups 3 and 4 were inoculated via the intraocular route with 0.05 ml medium containing 1×10^5 median EID_{50} of IBS037A/2014 at the ages of 1- and 30-days old, respectively. Groups 5 and 6 were controls for the ages of 1 and 30 days, respectively.

After virus inoculation, chickens in each group were observed and clinical signs were recorded daily. Thereafter, four chickens in each infected group and three chickens in each control group were selected randomly and killed in order to measure inhibition of ciliary activity in the trachea and to evaluate gross lesions at 2, 4, 6, 8, 12, 16, 20, 24 and 30 days post inoculation (dpi). At post-mortem examination, tissue samples from trachea, lungs, proventriculus, jejunum, caecal tonsil and kidney were collected to evaluate microscopical lesions, tissue tropism and virus load. Blood samples were also collected before death for serological studies.

Ciliostasis Testing

The ciliostasis test was performed as described by Yan *et al.* (2016) with some modifications. Briefly, the