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Pathogenic characteristics of Marek's disease virus field strains prevalent in China and the effectiveness of existing vaccines against them

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ABSTRACT

The virulence of Marek's disease virus (MDV) is continuously evolving, and more virulent MDV pathotypes are emerging, thereby reducing the effectiveness of the existing vaccines. In this study, feather pulps were collected from diseased chickens in commercial chicken flocks in China that presented significant MD visceral tumors in 2011 and were inoculated into a monolayer of duck embryo fibroblasts (DEFs). Three field isolates of MDV were obtained by plaque cloning and identified as MDV via PCR and designated strains LCC, LLY, and LTS. Unvaccinated and CVI988 vaccine-vaccinated specific pathogen-free chickens were challenged at 7 days post vaccination (dpv) with 1000 plaque forming units of each of the respective MDV isolates. These strains induced gross MD lesions in all (100%) of the unvaccinated chickens, and the mortality rates of the unvaccinated chickens were 42.9%, 46.7%, and 23.1% by 60 days post challenge (dpc), respectively. The CVI988 vaccine induced protective indices (PIs) of 85.7, 92.3, and 66.7, respectively. These results showed that the pathogenic characteristics of the Chinese isolates were diverse and that vaccine CVI988 provided different levels of protection against them. These data indicated that the existence of variant MDV strains was a possible reason of immunity failure in China.

1. Introduction

Marek's disease virus (MDV) is an oncogenic Alphaherpesvirus that belongs to the genus Mardivirus in which three closely related but distinct species have been grouped: MDV (gallid herpesvirus type 2, GaHV-2), which is the type strain for the genus; GaHV-3 (previously referred to as MDV-2); and turkey Herpesvirus 1 (HVT; meleagrid herpesvirus type 1, MeHV-1; previously MDV-3) (Osterrieder et al., 2006). Only MDV causes tumors in susceptible chickens, whereas the other two species are non-pathogenic (Witter and Schat, 2003). MDV strains are further classified into four pathotypes: mild (m), virulent (v), very virulent (vv), and very virulent plus (vv+) (Witter, 1983, 1997; Witter et al., 2005). MDV has become substantially more virulent over the last few decades. Unless more sustainable strategies for its control are implemented, it is likely that we will soon witness further shifts in its virulence and the emergence of more virulent strains (Nair, 2005).

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Marek's disease (MD) is a major disease caused by MDV that affects poultry health worldwide. MD vaccines are incapable of inducing a sterile immunity and allow the virulent virus strains to replicate and be shed into the environment through the vaccinated host. By keeping the host alive, vaccination prolongs the infectious periods of virulent strains and leads to the evolution of increased virulence (Atkins et al., 2013).

In recent years, the isolation and sequence analysis of MDVs in China were frequently reported in breeder or layer flocks that had been vaccinated with HVT or CVI988, and all of the results showed the existence of MDVs circulating in China (Teng et al., 2011; Yu et al., 2013; Zhang et al., 2011; Tian et al., 2011). However, the pathogenicity of MDVs circulating in China remains unclear. The isolation and culture of MDV field strains are essential for monitoring changes in MDV field strains and for the evaluation of the effectiveness of existing vaccines (Tan et al., 2008). Therefore, three MDV strains, denoted LCC, LLY, and LTS, were isolated from the feather pulps of diseased chickens with suspected MDV infection, the pathogenicity changes were monitored, and the effectiveness of the existing vaccines were evaluated in this study.

2. Materials and methods

2.1. Clinical samples

Several severe MD cases were described in layer flocks in the Liaoning and Jilin Provinces of China in 2011. The mortality rates of the flocks ranged from 5% to 40%, and the dead chickens showed visible tumors in the visceral organs. Feather pulps were collected from the dead or diseased chickens for diagnosis and viral isolation.

2.2. Viral isolation and identification

The viral isolation was performed as previously described (Zhang et al., 2011). Briefly, primary and secondary DEF and CEF cultures were prepared from 10-day-old SPF-embryonated duck or chicken eggs, which were obtained from the Harbin Veterinary Research Institute, as previously described (Schat and Purchase, 1998). Pooled feather pulps were suspended in SPGA/EDTA (sucrose, phosphate, glutamate, and albumin/ethylene diamine tetra-acetic acid) buffer for the extraction and titration of cell-free MDV. This suspension was ultrasonicated and then filtered through a 0.45-μm membrane filter for inoculation onto 24-hour-old duck embryo fibroblasts (DEFs). Blind passages were performed until a cytopathogenic effect (CPE) was observed. The infected cells were plaque-cloned, and the virus was propagated in the CEF cultures, harvested in M199 supplemented with 20% fetal bovine serum and 10% DMSO, and stored in liquid nitrogen.

The isolates were identified by PCR amplification of the genomic 132-bp repeat sequence (132bpr). The PCR-amplified 132bpr is unique in MDV and can be used to distinguish field MDV strains from the vaccine CVI988 strain (Silva, 1992).

2.3. Screening of adventitious agents

PCR and ELISA (using p27 mAb) methods were used to test Avian leucosis virus (ALV) (Gopal et al., 2012; Yun et al., 2013). The ALV HLJ09SH01 strain, which was isolated from China (Wang et al., 2012), was used as positive control for amplification of ALV in this study.

PCR and IFA (using gp90 mAb) methods were used to test Reticuloendotheliosis Virus (REV) (Gopal et al., 2012; Xue et al., 2012.). The REV HLJR0901 strain was isolated from China (Jiang et al., 2014.) and was used as a positive control for amplification of REV in this study.

The PCR method was used to test the Chicken infectious anemia virus (CIAV) (Qin et al., 2010) and CIAV M9905 strains (Wang et al., 2007), which were used as a positive control for amplification of CIAV.

The primers that were used for PCR amplification of MDV, ALV, REV, and CIAV are shown in Table 1.

2.4. Virulence studies

The CVI988/Rispens vaccine used in this study was a commercial vaccine. The infectious titer of the CVI988/Rispens vaccine and MDV isolates was determined by secondary CEF cultures (Table 2).

Table 1
Primers used to detect MDV, ALV, REV and CIAV.

Primer	Position	Sequence 5′ → 3′	Product size	Amplification target
132bp F	127553–127574[a]	TGCGATGAAAGTGCTATGGAGG	316–844[e]	MDV
132bp R	127848–127869[a]	GAGAATCCCTATGAGAAAGCGC		
p27 F	5258–5277[b]	GGATGAGGTGACTAAGAAAG	545	ALV
p27 R	5783–5802[b]	CGAACCAAAGGTAACACACG		
LTR F	101–118[c]	GCCTTAGCCGCCATTGTA	383	REV
LTR R	465–483[c]	CCAGCCAACACCACGAACA		
VP2-F	380–398[d]	ATGCACGGGAACGGCGGAC	650	CAV
VP2-R	1013–1030[d]	TCACACTATACGTACCGG		

[a] 132bp F and 132bp R, primers were derived from the MDV GA sequence (GenBank accession No. AF147806).
[b] p27F and p27R, primers were derived from the ALV HPRS-103 sequence (GenBank accession No. Z46390).
[c] LTR F and LTR R, primers were derived from the REV APC-566 sequence (GenBank accession No. DQ387450).
[d] VP2-F and VP2-R, primers were derived from the CIAV Cux-1 sequence (GenBank accession No. M55918).
[e] Exact size depends on the copies of 132bpr of MDV strains.