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不同H9N2亚型禽流感病毒分离株致病力研究及HA抗原性变异分析

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Characteristics of Pathogenic and HA Antigenic Variation of H9N2 Subtybe Avian Influenza Viruses Isolated from 1998 to 2008 in China

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摘要 【目的】了解近年来中国H9N2亚型禽流感病毒毒力变化和抗原性变异的特点，【方法】对分离于1998—2008年间的25株H9N2亚型禽流感病毒分离株进行了EID50、ELD50、MDT、ICPI、IVPI和8周龄SPF鸡人工感染排毒试验，测定了部分分离株与抗H9N2亚型禽流感病毒Hp参考株HA蛋白单抗2A4和F6的血凝抑制（HI）和中和反应特性，对具有不同反应特性分离株的HA基因进行了序列分析。【结果】不同分离株呈现致病力差异，具多态性特征，3#、12#和14#分离株致病力偏强，能引起部分SPF鸡发病和死亡，人工感染8周龄SPF鸡排毒时间更早，排毒期更长。3#和12#分离株与单抗2A4和F6呈现特殊的反应特性，单抗不能抑制3#和12#的血凝特性，也不能中和病毒感染CEF细胞。HA蛋白氨基酸序列分析表明，3#和12#分离株145位氨基酸发生漂变（S→N），导致与单抗的血凝抑制反应特性丢失，说明该位点（S145）为H9N2亚型禽流感病毒HA蛋白的一个抗原表位，是血凝抑制抗体结合位点。S145N的漂变导致在145—147位氨基酸多出一个糖基化位点NGT，可能是分离株毒力增强的原因。【结论】本研究结果表明，H9N2亚型禽流感病毒呈现变异趋势，出现了有致病力和抗原性变异流行毒株。S145为H9N2亚型禽流感病毒HA蛋白的一个抗原表位，但有该位点漂变导致的抗原变异毒株出现，并可逃避免疫作用，对该病的防控提出了新的挑战。

关键词: 禽流感病毒 H9N2亚型 HA蛋白 致病力 抗原变异

Abstract: 【Objective】 The objective of this experiment is to investigate the characteristics of pathogenic and antigenic variation of H9N2 subtybe avian influenza viruses isolated from 1998 to 2008 in China. 【Method】 The EID50, ELD50, MDT, ICPI, IVPI and the duration of shedding virus from infected 8-week-old SPF chickens of different H9N2 avian influenza viruses isolates were determined. The HI and VN activity of monoclonal antibody 2A4 and F6 on different H9N2 avian influenza viruses isolates were assayed and the HA genes of different antigenic reactive isolates were sequenced and analyzed. 【Result】 The determined pathogenicity suggested that the virulence of different isolates were different, thereinto, 3#, 12#, and 14# isolates showed higher pathogenicity than the others and could cause the death of SPF chickens. The 8-week-old SPF chickens infected by 3# or 12# isolate shed virus earlier and last for a longer time. 3 # and 12 # isolates showed specific response properties to monoclonal antibody 2A4 and F6. Monoclonal antibody 2A4 and F6 could inhibit the hemagglutinin activity of 3 # and 12 # isolates, however it could not neutralize the virus infection on CEF cells. HA sequence analysis showed that there was a single amino acid substitution of Ser (S)-to- Asn(N) at position 145 in the HA protein of 3 # and 12 # isolates, which led to the loss of reactivity to the monoclonal antibody 2A4 and F6 and the occurrence of a new potential glycosylation site NGT. The change of reactivity to the monoclonal antibody 2A4 and F6 suggests that the site (S145) is one of HA protein epitope of the H9N2 subtype avian influenza A virus. The new occurring potential glycoprotein site NGT in the HA protein of 3 # and 12 # isolates may cause the enhancing pathogenicity. 【Conclusion】 The results show that H9N2 subtype avian influenza virus isolates have the tendency to evolve, resulting to the occurrence of mutants which have higher virulence and variable antigenicity. The higher virulent mutants may cause death of chickens and lead to more economic loss. The antigenic mutants may evade the immunity, which pose a new challenge to the immune prevention for the H9N2 avian influenza.

Key words: avian influenza virus H9N2 subtype HA glycoprotein pathogenicity antigenic variation

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