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Piglets and Chicken Artificially Infected by Newcastle Disease Virus with Different Virulences

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Abstract: To determine the capability for infection and pathogenicity of Newcastle Disease Virus (NDV) in pigs and chicken, researchers used two NDVs (Xiny from pigs and virulent strain F48E9) to infect weaned piglets and Specific Pathogen-Free (SPF) chicken artificially. Results showed that strains F48E9 and Xiny were inadequately virulent to cause piglet illness or death. However, the virus agents are detectable in the pig body. F48E9 can cause the death of SPF chickens but Xiny caused no pathology in such chickens.

Key words: Newcastle disease virus, pig, SPF chicken, pathogenicity, interspecies transmission

INTRODUCTION

Newcastle Disease (ND) Virus (NDV) is a severe infectious avian pathogen that can cause serious economic losses. Over the past several years, cases of ND have frequently appeared in China (Yu *et al*., 2001; Liu *et al*., 2007). NDV was previously considered to infect only avian species. However, pig-origin NDVs have increasingly been reported in recent years (Chen *et al*., 2013; Ding *et al*., 2010). To determine the capability of NDV for interspecies transmission in pig and chicken, researchers artificially infected piglets and Specific Pathogen-Free (SPF) chicken with NDV strains of different virulences.

MATERIALS AND METHODS

NDV strains: Pig-origin Xiny was isolated and stored in the Shandong Academy of Agricultural Sciences. Virulence detection showed that this strain has low virulence and might be generated from La Sota through genetic and biological characterizations (Yuan *et al*., 2012). Chinese-standard virulent strain F48E9 was purchased from Control Institute of Veterinary Bioproducts and Pharmaceuticals, China. All strains were subjected to passaged rejuvenation using 10 days SPF chickens and then kept at -20°C for use after the ELD_{50} or EID_{50} measurement of each strain.

Attacking piglets and SPF chicken: The 4 weeks old weaned piglets were from the Shandong YF farming company. The 4 weeks old SPF chickens were from the Shandong Institute of Poultry Science. ND vaccination was not performed during artificial feeding and the pigs and chickens were fed normally. Pig and chicken serums were collected to measure NDV antibody levels using a hemagglutination-inhibition assay. NDV-positive serums and antigens were provided by Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences.

Attacking test: Fifteen piglets and fifteen SPF chicken were randomly divided into three groups: five for the Xiny infection group, five for the F48E9 infection group and the remaining for the saline control group. Each group was fed separately. Neck muscle inoculation was performed with a dosage of 10^6 ELD_{50} 0.2 mL^{-1} or 10^6 EID_{50} 0.2 mL^{-1}.

After infection, changes in body temperature were measured daily. Throat and cloacal cotton swabs were collected from each group every 5 day. Polymerase Chain Reaction (PCR) and virus isolation were used to determine whether a virus existed.

PCR test after attacking: Primer Premier 6.0 Software was used to design a pair of primers as follows: JC-F: 5'-CACTCAAATATGGAATCAATAC-3', JC-R: 5'-GTC ACTGTCACCTAATAATG-3' to amplify the 146 bp fragment according to the F gene sequence of Xiny (Genbank No.: JN032760). Primers were provided by TaKaRa Biotechnology (Dalian) Co., Ltd. and PAGE purification was used for primer synthesis. A TaKaRa One-step RNA PCR Kit (AMV) was used for the real-time PCR reaction. The reaction procedure was as follows: 50°C for 50 min, pre-denaturation at 95°C for 3 min; denaturation at 94°C for 30 sec; annealing at 50°C for 30 sec; an extension at 72°C for 30 sec, totaling 35 cycles;

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an extension at 72°C for 5 min and finishing at 4°C. After amplification, the reaction solution was taken and subjected to agarose gel electrophoresis. Samples were observed to determine the presence of a 146 bp fragment.

RESULTS

In the pig infection test, pigs subjected to pig-origin strain Xiny and virulent strain F48E9 were observed 15 days after infection and no deaths were recorded. After dissection, no pathological change in the liver, lungs and other organs was observed in the two challenge groups (Fig. 1). In the chicken infection test, F48E9 can result in the death of SPF chickens. After autopsy, the typical NDV pathological changes can be observed. However, Xiny did not cause any illness or death in SPF chickens, similar to the case with piglets.

Changes in the body temperature of each piglet are shown in Fig. 2. The changes among the piglets were

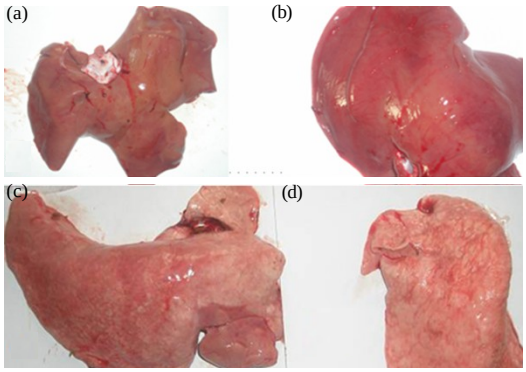

Fig. 1: Liver and lung samples obtained after swine infection; a) liver of the infected group; b) liver of control group; c) lung of infected group; d) lung of control group; No pathological change in the liver or lungs was found in the infected and control groups

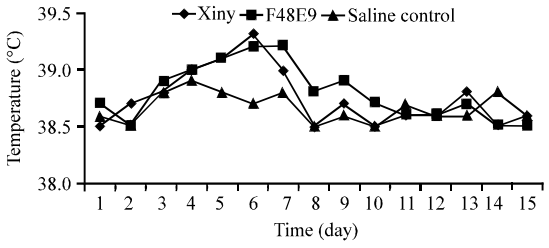

Fig. 2: Changes in the body temperature of infected piglets. Only approximately 6-8 day after the infection test did body temperatures increase slightly

low. After approximately 6-8 days after the infection did body temperatures increase slightly. This increase in temperature may have been caused by the body immune reaction. No swelling, fever or other inflammatory reaction was observed around the injection site.

Throat and cloacal cotton swabs were collected every 5 days (for a total of three collections) for pathogen detection. Researchers found that NDVs (Xiny and F48E9) can be detected in the majority of samples.

DISCUSSION

Hosts infected with NDV have gradually increased in recent years (Cai *et al*., 2011; Hoque *et al*., 2012; Sharma *et al*., 2012). NDVs isolated from pigs are worthy of considerable attention. This study illustrates the capability of NDV for cross-species infection by simulating the pathogenicity of NDV in pigs or chickens in the natural state. In the study, virulent strain F48E9 can cause death in SPF chickens but when used to artificially infect 4 week old piglets, no symptoms of death or pathological changes were observed. These findings indicate that virulent NDV cannot adapt to pig hosts and thus cannot cause death. Meanwhile, Xiny as an avirulent NDV strain, similarly cannot induce any change in piglets.

CONCLUSION

In future research in-depth investigations on the interactive pathogenicity of different host strains may be conducted based on the enhancement of studies on pig-origin NDV epidemiology and ecology.

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