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Short communication

The distribution of sialic acid receptors of avian influenza virus in the reproductive tract of laying hens

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ABSTRACT

The susceptibility of the host to influenza virus is determined by the distribution of the sialic acid (SA) receptors on host cell membrane. Avian influenza virus (AIV) preferentially binds to SA α-2,3-galactose (SA α2,3-gal) linked receptors, while human strains bind to sialic acid α2,6-galactose (SA α2,6-gal) linked receptors. Here, we describe the SA patterns and distributions in the reproductive tract of hens by employing two specific lectins, Maackia amurensis agglutinin (MAA) for SA α2,3-gal and sambucus nigra agglutinin (SNA) for SA α 2,6-gal receptors. Our results revealed that both SA α2,3-gal and SA α2,6-gal receptors exist in the reproductive tract of hens, including magnum, isthmus, uterus and vagina except for infundibulum. The distribution of SAα-2,3-gal receptor was more abundantly in the columnar epithelium cells of magnum, isthmus and uterus. Only minimal positive results for SA α-2,6-gal receptors were detected in the columnar epithelium cells of magnum, isthmus, uterus and vagina. Furthermore, AIV in tissues of the reproductive tract tissues of laying hens were detected by SYBR green-based reverse transcription and polymerase chain reaction (RT-PCR). Results showed that both viral loads and pathological changes in different parts of the reproductive tract were positively correlated with the expression of both receptors. Our results revealed that the reproductive tract of hens may provide an environment for the replication of both avian and human influenza viruses.

Avian influenza virus (AIV) is an important infectious agent in a wide variety of domestic poultry, captive birds, and free-ranging wild bird species under natural and experimental conditions, posing a major threat to animal health as well as a zoonotic threat to humans [1,2]. AIV generally infects poultry because the specific structure of its hemagglutinin (HA), a viral attachment protein for host receptor binding. The genetic reassortment of AIV occurs during their replications to obtain the ability of infecting different species or humans. For instance, H5N1 and H7N9 could not only trigger huge economic losses in poultry industry but also cause human casualties [3,4]. Based on the clinical symptoms in chickens, AIV was classified as low pathogenic avian influenza virus (LPAIV) and highly pathogenic avian influenza virus (HPAIV). Typically, LPAIV caused asymptomatic infections in wild aquatic birds, but clinical signs and lesions were presented when domesticated poultry were infected with LPAIV, and then caused pathological damages to the respiratory [5], digestive and reproductive systems, such as subclinical disease or mild respiratory signs with severe depression in egg production [6,7]. The hens, which infected with H9N2 AIV, egg production could drop

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precipitously [8]. To initiate the infection process, the HA of influenza virus must firstly bind to SA receptors and then promote the receptors-mediated endocytosis. The capability of receptors-binding will determine the host range of influenza viruses. AIVs preferentially bind to SA α2,3-Gal receptors, while human strains bind to SA a2,6-Gal receptors [9,10].

Previous studies have suggested the expression of SA receptors in human, pig, quail, duck, pheasant and chicken [10,11]. AIV has been known to induce a decrease in egg production and egg quality [12], and the virus has been detected on the eggshell and also in internal components of the egg and in the reproductive tract after natural infection [13], indicating that the virus replicates in the reproductive tract. The oviduct is an independent organ in laying hens which has a certain influence on AIV transmitting through the eggs. Since the SA receptors are critical for the replication of AIV, we hypothesized that the expression distribution of SA in the oviduct of hens may play an important role in the pathogenesis and egg transmission of AIV. However, very little is known regarding the SA receptors distribution in the oviduct and the reproductive tract of laying hens. To study the type and distribution of SA receptors and the existence of AIV in the reproductive tract (infundibulum, magnum and isthmus, uterus, and vagina) of laying hens, lectin histochemistry, SYBR green-based real-time reverse transcription-polymerase chain reaction (RT-PCR), and pathological examination were employed in this study. Our findings reveal that both SA α2,3-gal and SA α2,6-gal receptor expression in the magnum, isthmus, uterus, and vagina positively correlated with the presence of AIV in these tissues.

In this study, a total of 20 SPF White Leghorn hens were supplied by the Yang Ling Green Biological Engineering Co. and housed in isolators under negative pressure with food and water provided ad libitum. At the age of 25 weeks, the hens were randomly divided into two groups. The first group having 10 hens was examined for the expression of SA receptors using lectin histochemistry and served as uninoculated controls in HE staining. The second group of ten hens was inoculated with a H9N2 AIV strain (A/chicken/Shaanxi/11/2012(H9N2)) [14] by a combined intraocular and intranasal route using a total dose of 10^6 median embryo infective doses [15]. Initially, the clinical signs of viral infection were observed by 3 days post-inoculation (dpi), as most hens experimentally infected with the virus were depressed, consumed less food and water, 7 hens displayed poor quality of eggs. By 5 dpi, 9 hens sacrificed and the last one hen could not stand up. By 7 dpi, the last one hen sacrificed. All the hens were euthanized by cervical dislocation under conditions strictly in keeping with international standards of animal welfare. Throughout the study, the laying hens were handled according to an approved Institutional Animal Care and Use Committee guideline. All animal experimental procedures were approved by the Ethical and Animal Welfare Committee of Shaanxi Province, China. In this work, tissue samples of the infundibulum, magnum, isthmus, uterus and vagina were collected for this study. The half tissues of second group were preserved in liquid nitrogen promptly for SYBR green-based real time RT-PCR. The first and half tissues of second groups were fixed in 10% neutral buffered formalin for 24 h, then fixation in fresh 10% neutral buffered formalin for another 24 h [2,11]. Following fixation, tissues were dehydrated, embedded and sectioned. Tissue sections were 5 microns thick (Leica Biosystems, Germany).

Tissue sections were deparaffinized with xylene for 10 min and sequentially hydrated with 100%, 95%, 90%, 80% and 70% alcohol for 5 min at each step. The tissues were then blocked for nonspecific binding with 5% bovine serum albumin in phosphate buffer saline (PBS) for 40 min at 37 °C. After discarding blocking solution, sections were incubated with 1 mg of fluorescein isothiocyanate

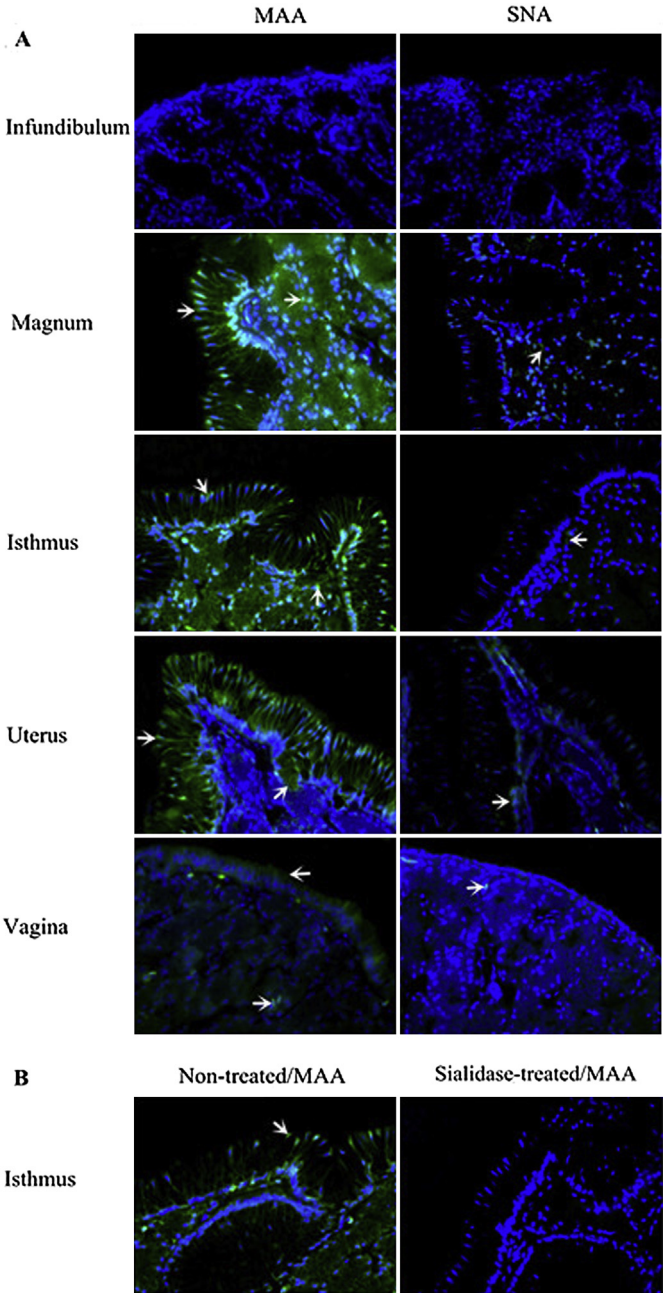

Fig. 1. Expression of both SA α-2,3-gal and SA α-2,6-gal receptors in each part of the reproductive tract of hens. Representative micrographs of the reproductive tract of hens (infundibulum, magnum, isthmus, uterus, vagina) showing distribution of both SA α-2,3-gal and SA α-2,6-gal receptors. Tissue sections were stained with FITC conjugated lectin MAA (left panel) or SNA (right panel), specific toward SA α-2,3-gal and SA α-2,6-gal receptors, respectively (A). To confirm the specificity and the presence of SA α-2,3-gal in the reproductive tract of hens, the tissue section of isthmus were digested with 1 U/ml of sialidase before MAA staining. The sialidase-treated tissue (panel B, right) lost the staining signal on the apical surface, while non-treated section was positive (Panel B, left) (B). Both SA α-2,3-gal and SA α-2,6-gal receptors were expressed in the reproductive tract oviduct of hens except infundibulum. The SA α-2,3-gal receptors was prevalent in the magnum, isthmus, uterus and vagina, especially on the columnar epithelium cell. In the magnum, isthmus and uterus, the SA α2,3-gal receptors was diffuse distribution. On the contrary, SA α-2,6-gal receptors was infrequent in the tubular glands of magnum, isthmus, uterus and vagina. The arrows indicate the positive parts. ×400.