Susceptibility of turkeys, chickens and chicken embryos to SARS-CoV-2 virus

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Abstract

Susceptibility of turkeys, chickens and chicken embryos to SARS-CoV-2 virus was evaluated by experiment inoculation. Turkeys and chickens were inoculated using a combination of intranasal, oral and ocular routes. Both turkeys and chickens did not develop clinical disease or antibodies to the virus following inoculation. Viral RNA was not detected in oral and cloacal swabs and in tissues using quantitative real-time RT-PCR. In addition, chicken embryos were inoculated using the yolk sac, intravenous, chorioallantoic membrane and allantoic cavity routes did not support replication of the virus. SARS-COV-2 virus does not affect both turkeys and chickens in the current genetic state and does not pose any potential risk to establish in both species of domestic poultry.

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Running Title: Susceptibility of poultry to SARS-CoV-2 virus

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Abstract

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KEYWORDS

SARS-CoV-2; Susceptibility; Chicken Embryos; Turkeys, Chickens; COVID-19

1 Introduction

The coronavirus disease (COVID-19) outbreak starting in 2019 was declared as public health emergency by the World Health Organization (WHO) on January 30, 2020, infecting more than 16.5 million people and continuing to spread throughout the world (WHO 1a, 2020; WHO 1b, 2020). The disease COVID-19 was first described in Wuhan, China, in patients that had respiratory tract infection and pneumonia (Zhou et al., 2020; Andersen et al., 2020). It is caused by a novel betacoronavirus known as severe acute respiratory syndrome virus 2 (SARS-CoV-2) that is genetically related to bat coronavirus RaTG13 and other betacoronaviruses that are found in bats (Zhou et al., 2020; Andersen et al., 2020; Lu et al., 2020).

Viruses in the coronavirus family are enveloped, single stranded positive sense RNA viruses with genomes ranging between 26 to 32 kb (Su et al., 2016; Fehr et al., 2015). The coronavirus family contains a wide range of viruses that are pathogenic to a variety of animal species including humans. However, many of these viruses tend to have limited host range and can cause severe diseases only in domestic animals, resulting in large production losses to producers. Some animal coronaviruses such as transmissible gastroenteritis virus (TGEV), bovine coronavirus (BCoV), feline coronavirus, canine coronavirus and turkey coronavirus cause enteritis in their respective hosts (Su et al., 2016). Others such as avian infectious bronchitis and canine respiratory coronavirus cause respiratory diseases in their respective hosts (Su et al., 2016).

Prior to 2003, viruses of the coronavirus family were not known to cause severe disease in humans. However, since the beginning of the 21st century three viruses from the betacoronavirus subfamily, Severe Acute Respiratory Syndrome virus (SARS), Middle Eastern Respiratory Syndrome virus (MERS) and SARS-Cov-2 have crossed the species barrier to infect humans (Kin et al., 2015; Peiris et al., 2003; Zhou et al., 2020).

When a novel virus results in an outbreak or pandemic, assessing the risk of the novel agent to infect other species is of paramount importance in order to control the disease. Since the emergence of SARS-CoV-2, a number of studies have been conducted as part of the risk assessment to determine if domestic animals that are used as companion animals in households or as source of food are susceptible to the virus. Additional studies were also conducted in laboratory animals such as mice, ferrets, non-human primates and others in search of animal models that could be used to study virus pathogenesis, vaccine efficacy studies and prophylaxis of the disease.

The purpose of this study was to determine if domestic poultry such as turkeys and chickens are susceptible to SARS-CoV-2 virus and play any potential role in the dissemination of the virus. In addition, the susceptibly of chicken embryos to SARS-CoV-2 was evaluated to determine if eggs could potentially be used for the production of potential SARS-CoV-2 vaccines.

2 Materials and Methods

2.1 Animal Experiment

The animal experiments were conducted under the approval of the Canadian Science Center for Human and Animal Health Animal Care Committee which follows the guidelines of the Canadian Council on Animal Care. To determine if domestic poultry are susceptible to SARS-CoV-2 infection, fourteen 4- to 6-week-old leghorn chickens (*Gallus gallus domesticus*) were acquired from the CFIA SPF flock in Nepean, Ottawa and fourteen 4-to 6-week-old turkey (*Melleagris gallopavo*) pullets were purchased from a local turkey breeder in Manitoba. Both turkeys and chickens were kept for a week in biosafety level 3 animal cubicles for acclimatization for a week. After a week, both turkeys and chickens were split into 2 groups (SARS-CoV-2 and control). Prior to infection, blood, oropharyngeal and cloacal swab samples were collected from SARS-COV-2 and control group chickens. Ten chickens and 10 turkeys were inoculated using a combination of natural routes of infection (combination of oral, nasal and ocular) with one ml containing 10^6 plaque forming units per ml (PFU/ml) of SARS-COV-2 virus. Control groups were mock inoculated using same routes with PBS. Oropharyngeal and cloacal swabs were collected from infected and control groups at 2, 4, 6, 9 and 12 days post-inoculation. Two chickens and two turkeys were euthanized on 3, 5, and 7 days

post-inoculation and tissues samples were collected (brain, thymus, trachea, lungs, spleen, duodenum, cecal tonsil, liver, kidneys, bursa and heart).

2.2 Chicken Embryo Susceptibility Study

To determine if chicken embryos were susceptible to SARS-CoV-2 virus infection, chicken embryos were inoculated by different routes commonly used to virus isolation. Six day old chicken embryos were inoculated using the yolk sac route; 9 day old chicken embryos inoculated in the chorionallantoic membrane (CAM) and the allantoic cavity; and 11 day old chicken embryos inoculated by the intravenous route. The embryos were candled twice daily to monitor for mortality. Two successive blind passages were performed and after the 1st and 2nd passages, total RNA was extracted from the amion allantoic fluid (AAF), embryo and CAM and checked for the presence of SARS-CoV-2 genomic material using the E-gene specific RT-qPCR.

2.3 RNA Extraction

Total RNA extraction from all clinical samples was conducted using the MagMAX Express-96 Magnetic Particle Processor (Thermo-Fisher) using the MagMax CORE Nucleic Acid Purification Kit (Thermo-Fisher) with some modification. Samples were inactivated with tripure reagent (1:9 ratio). Then, 650 µL of the inactivated sample, 30µL of binding beads, and 350 µL binding buffer spiked with enteroviral armoured RNA (ARM-ENTERO; Asuragen) was used for extraction. The extracted RNA was eluted in 30 µL elution buffer. The spiked enteroviral armoured RNA was used as an exogenous RNA extraction control.

2.4 Real-time RT-PCR

Total RNA was extracted from all clinical samples and checked for the presence of SARS-Cov-2 RNA by an E gene specific semi quantitative real-time RT-PCR assay (RT-qPCR) that detects a broad range of human and bat coronaviruses (Corman et al., 2019). For the detection of SARS-CoV-2 RNA by RT-qPCR, we used 4X TaqMan® Fast Virus one step RT-PCR kit (Life Tech., USA) according to manufacturer's recommendations. For each RT-qPCR reaction, 0.4 µM of each E gene forward and reverse primers and 0.2 µM of probe were used. RT-qPCR runs were performed using a 7500 Fast Real-Time PCR System (Applied Biosystem) using the following cycle conditions: 50°C for 5 min, 95°C for 20 sec, and 40 cycles of 95°C for 3s followed by 60°C for 30s. RT-qPCR semi-quantitative results were calculated based on a gBlock (Integrated DNA Technologies, IDT) standard curve for SARS-CoV-2 E gene.

2.5 Virus Neutralization Assay

To determine the presence of neutralizing SARS-CoV-2 antibodies in the sera of chickens and turkeys, a plaque reduction neutralization test was used. Serum samples collected at 0, 7, 14 and 21 days after infection were heat inactivated at 56°C for half an hour. Serial five-fold dilutions of the inactivate sera were incubated with virus for 1 hour at 37°C. Each virus-serum mixture was then added to duplicate wells of Vero E6 cells in a 48-well format, incubated for 1 hour at 37°C, and overlaid with 500 µl of 2.00% carboxymethyl cellulose (Sigma) in Dulbecco's Modified Eagle Medium (Thermo-Fisher) per well. Plates were then incubated at 37°C for 72 hours, fixed with 10% buffered formalin and stained with 0.5% crystal violet. Serum dilutions resulting in >70% reduction of plaque counts compared to virus controls were considered positive for virus neutralization.

2.6 Histopathology

Tissue samples (brain, thymus, trachea, lungs, heart, kidneys, pancreas, gut, cecal tonsil and liver) were fixed in formalin. After 48 hrs, the fixed tissues samples were embedded in paraffin, sectioned, stained with H & E and were examined for microscopic lesions as described previously (Neufield et al., 2009).

3 Results and Discussion

Chickens and turkeys did not develop any clinical signs of the disease for the entire interval of the experiment. No shedding of SARS-COV-2 genomic material was detected in cloacal and oropharyngeal swabs collected

from both turkeys and chickens at 2, 4, 6, 9 and 12 days post-inoculation by RT-qPCR. In addition, no SARS-COV-2 genomic material was detected in tissues (brain, thymus, trachea, lungs, heart, kidneys, pancreas, gut, cecal tonsil and liver) collected from chickens or turkeys at 3, 5 and 7 days after infection by RT-qPCR.

No gross pathological and histopathological changes were observed in the brain, thymus, trachea, lungs, heart, kidneys, pancreas, gut, cecal tonsil and liver. No SARS-COV-2 specific antibodies were detected in serum samples collected from both chickens and turkeys at 7, 14 and 21 days after infection using the plaque reduction neutralization test.

Chicken embryos inoculated with SARS-COV-2 using the different routes were alive 6 days after inoculation. Pools of embryo tissues, AAF and CAM were harvested and checked for presence SARS-CoV-2 genomic material by RT-qPCR. After the first passage, we were able to detect presence of SARS-CoV-2 genomic material in the AAF, CAM and body of the infected embryos in ECEs inoculated using different inoculation routes. However, based on RT-qPCR results, the amount SARS-COV-2 genomic material in all ECE samples analyzed was almost a log lower when compared to the RT-qPCR results of the original inoculum indicating that this is residual genomic material SARS-CoV-2 from the inoculum (Fig. 1A). In normal situations, if the virus is replicating in embryos you will see increase in the presence of virus compared to the inoculum. Embryo samples from each inoculation group were used for conducting a 2^{nd}passage. After 6 days of incubation, no chicken embryo mortality was observed. Amion allantoic fluid was harvested from each group and evaluated for the presence SARS-CoV-2 RNA using the E-gene specific RT-qPCR (Fig. 1B). Only traces of genomic material of the virus were detected in all samples collected from chicken embryos inoculated using the different routes of inoculation indicating that chicken embryos are not susceptible to SARS-CoV-2.

To determine if the SARS-CoV-2 that was collected from 4 different embryo inoculation groups after 1^{st} and 2^{nd} passages was still viable, Vero E-6 cells grown to 90-95% confluence were inoculated with chicken embryo samples that had lower CT values based on the RT-qPCR. No cytopathic (CPE) was observed in Vero E-76 cells after 2 consecutive cell passages. This demonstrates that the virus that was detected in the embryo samples by RT-qPCR was not viable and was residual genomic material of the virus inoculum used to infect the embryos.

SARS-COV-2 virus does not affect both turkeys and chickens in the current genetic state and does not pose any potential risk to establish in these of species of domestic poultry. Previous study has shown that chickens infected with SARS-CoV-2 using the intranasal route were not susceptible to clinical infection (Shi et al., 2020). However, in the current study, we also demonstrate that turkeys, chickens and chicken embryos inoculated using different inoculation routes are not susceptible to SARS-CoV-2 infection. Unlike *Gammacoronavirus* and*Deltacoronavirus* genera of the Coronavirus family, viruses in betacoronavirus genus do not seem to infect birds (Woo et al., 2012). These findings are very important for risk analysis as chickens, turkeys and eggs are an important component of the human diet and are also found in large numbers in farms as well as in backyards of suburban population.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the Transboundary and Emerging Diseases Journal have been adhered to and that the animal experiments were conducted under the approval of the Canadian Science Center for Human and Animal Health Animal Care Committee which follows the guidelines of the Canadian Council on Animal Care.

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Figure Legends

Figure 1. Persistence of SARS-CoV-2 genomic material in AAF, CAM and body of the chicken embryos inoculated using different inoculation routes after 1 passage (Fig 1A) and 2nd passage (Fig 1B). RT-qPCR semi-quantitative results were calculated based on a gBlock (Integrated DNA Technologies, IDT) standard curve for SARS-CoV-2 E gene.