Polish Journal of Veterinary Sciences Vol. 21, No. 3 (2018), 491–495

DOI 10.24425/122621

Original article

Serological surveillance of avian influenza virus and canine distemper virus in captive *Siberian Tigers* in Northeastern China

K. Wang[1a], H. Wang[2a], N. Feng[3], H. Wang[3], Y. Zhao[3], Y. Gao[3]*, G. Hu[1]*, X. Xia[1,3,4]*

[1] College of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, P. R. China
[2] Wildlife ambulance breeding center of Jilin province, Changchun, Jilin, P. R. China
[3] Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Military Veterinary Research Institute of Academy of the Military Medical Sciences, Changchun 130122, P. R. China
[4] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, P. R. China

Abstract

In order to understand infection of avian influenza A virus (AIV) and canine distemper virus (CDV) in the *Siberian Tiger* in Northeast China, 75 *Siberian Tiger* serum samples from three captive facilities in northeastern China were collected. AIV and CDV antibody surveillance was tested by using hemagglutination inhibition and serum neutralization methods. The results showed that the seroprevalence of H5 AIV, H9 AIV and CDV was respectively 9.33% (7/75), 61.33% (46/75) and 16% (12/75). In the 1<years <2 and > 5 year-old group, the seroprevalence of the H9 AIV was 24% and 80% ($P < 0.01$), and the CDV seroprevalence was 6% and 36% ($P < 0.01$), respectively. It was demonstrated that 3 (4%) out of 75 serum samples were AIV+CDV seropositive, with 2.67% (2/75) in H9+AIV and 1.33% (1/75) in H5+H9+AIV. To our knowledge, this is the first report of AIV and CDV seroprevalence in *Siberian Tiger*s in China, which will provide base-line data for the control of AIV and CDV infection in *Siberian Tiger*s in China.

Keywords: influenza A virus, canine distemper virus, serological, Siberian Tiger

Introduction

Infection with avian influenza A virus (AIV) and canine distemper virus (CDV) has been reported to threaten the survival of endangered tigers as a newly emerging disease. Influenza A virus infection in tigers was first reported in 2002 (Xia et al. 2003), and H5N1 AIV was then identified from tigers that had died of respiratory distress in zoos (Mushtaq et al. 2008, Fukui et al. 2013, He et al. 2015). Furthermore, some cases of canine distemper in captive and wild tigers had been reported (Myers et al. 1997, Seimon et al. 2013,

Correspondence to: Y. Gao, G. Hu, X. Xia, e-mail: guixue1964@126.com (G Hu)
[a] These authors contributed equally to this work.

Table 1. Seroprevalence of avian influenza A virus (AIV) infection in *Siberian tiger* in northwestern China, by Hemagglutination (HA) and Hemagglutination inhibition (HI) assays.

Subtype	Changchun (%)	Harbin (%)	Shenyang (%)	Total (%)
H3	0 (0/31)	0 (0/29)	0 (0/15)	0 (0/75)
H5	3.23 (1/31)	17.24 (5/29)	6.67 (1/15)	9.33 (7/75)
H7	0 (0/31)	0 (0/29)	0 (0/15)	0 (0/75)
H9	35.48 (11/31)	72.41 (21/29)	93.33 (14/15)	61.33 (46/75)
H5+H9	0 (0/31)	13.8 (4/29)	6.67 (1/15)	6.67 (5/75)
Total	38.71 (12/31)	75.86 (22/29)	93.33 (14/15)	64 (48/75)

Table 2. Seroprevalence of canine distemper virus (CDV) infection in the *Siberian tiger* in northwestern China, by neutralizing antibody assays.

Factor	Changchun	Harbin	Shenyang	Total
CDV Negative	80.65% (25/31)	68.97% (20/29)	93.33% (14/15)	78.67% (59/75)
CDV Suspect	6.45% (2/31)	3.45% (1/29)	6.67% (1/15)	5.33% (4/75)
CDV Positive	12.9% (4/31)	27.59% (8/29)	0 (0/15)	16% (12/75)
H5+CDV	0% (0/31)	0% (0/29)	0% (0/15)	0% (0/75)
H9+CDV	3.23% (1/31)	6.90% (2/29)	0% (0/15)	4% (3/75)
H5+H9+CDV	0% (0/31)	3.45 (1/29)	0% (0/15)	1.33% (1/75)

Note: Negative (<1:8); Suspect (1:8-1:16); Positive (>1:16)

Rastogi et al. 2014). These findings indicate that the health of endangered tigers is being threatened by AIV and CDV. However, the systematic investigation of AIV and CDV in endangered tigers has not been conducted to date. To better understand the potential prevalence for the AIV and CDV transmission among tigers, we collected 75 serum samples from *Siberian Tigers* in three zoos in Northeastern China and the serum antibody of AIV and CDV in these tigers was assayed.

Materials and Methods

Study Area

Northeast China (40°-53° N, 120°-135° E), including Liaoning province, Jilin province and Heilongjiang province, has a total area of 18.74 square kilometres. The climate is characterized by a cold, long winter and warm, short summer.

Sample collection

Blood samples were collected between 2015 and 2016. A total of 75 blood samples were collected from the *Siberian Tigers* randomly (but not repeatedly) from three captive facilities in northeastern China (Table 1). None of the *Siberian Tigers* were injected with any other vaccines. Blood samples from the femoral vein were collected when healthy tigers which had not previously suffered any disease were anesthetized using ketamine. The serum was obtained through centrifugation at 1000×g for 5min, and was then separated and stored at -20°C until analysis. The protocol was approved by the State Forestry Administration of China and the Committee on the Ethics of Animal Experiments of the Jilin Agricultural University, China.

Hemagglutination (HA) and Hemagglutination inhibition (HI) assays

HA and HI assays were used as serological tests to detect the presence of influenza-specific antibodies in the serum. H3, H5, H7 and H9 subtypes of influenza viruses were selected for HI assays. The antigen of A/chicken/Anhui/1/2006 (H5N1, clade 2.3.4), and A/African starling/England/Q/983/79 (H7N1), A/chicken/Shanghai/10/01 (H9N2, clade Y280/G9) were acquired from the National Avian Influenza Reference Center of China. A/Baikal teal/Xianghai/XH-28C/2012 (H3N2) was isolated from wild birds by our laboratory. An HI titer ≥ 20 was considered seropositive due to a previous infection. To eliminate any nonspecific inhibitory factors of agglutination, all serum samples were

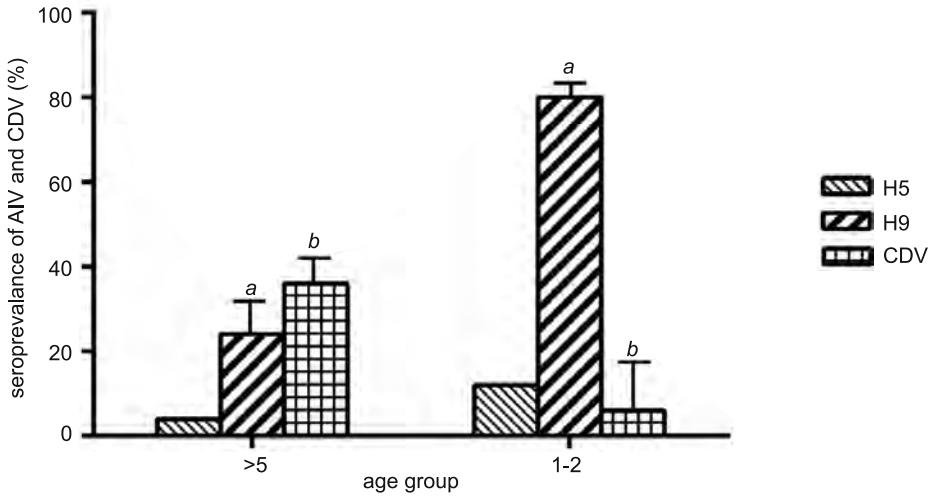

Fig.1. Seroprevalence of avian influenza A virus (AIV) and canine distemper virus (CDV) infection in the various age groups of the *Siberian tigers* in northwestern China. The significance analysis was marked with the symbol "a, b" ($p<0.01$).

treated with a receptor-destroying enzyme (RDE) and absorbed by the erythrocytes. RDE was added to the sera at 37°C overnight followed by incubation at 56°C for 1 h to inactivate the RDE (Yu et al., 2015). The serum samples were then incubated with erythrocytes for 22 min at 4°C and centrifuged at 500 g for 10 min to remove the erythrocytes. The HI tests were performed according to the World Organization for Animal Health recommendations. In brief, 25 μL of serial two-fold dilutions of the treated serum samples were mixed with 4 HA units of the virus in microtiter plates and incubated at room temperature for 22 min. 25 μL of 1% chicken erythrocytes were then added to each well and incubated at room temperature for 40 min. The HI titer was calculated as the reciprocal of the highest serum dilution that completely inhibited virus-mediated hemagglutination.

CDV-neutralizing antibody assay

Antibody titers to CDV were measured via virus neutralization using Vero cells as previously described (Kimber et al., 2000). The antigen for the CDV assay was the CDV/R-20/8 vaccine strain. The intended starting dilution for these assays is 1:4. Aliquots of virus (100 $TCID_{50}$) were incubated at 37°C for one hour with serial dilutions of the test or control sera (heat inactivated at 56°C for 30 min) and were then added to Vero cells growing in a flat-bottomed 96-well tissue culture plate. Cells were observed microscopically for CPE starting on day 3 post-infection. The CPE recorded at day 5 was used for the calculation of CDV virus neutralization (VNA) titers. The virus neutralization titer was defined as the highest serum dilution that inhibited CPE.

Statistical Methods

All statistical analyses were performed using PASW Statistics 19.0 software (SPSS Inc., IBM Corporation, Somers, NY). Results were considered statistically significant when $p<0.05$.

Results

AIV exposure in captive tigers

It was demonstrated that 48 (64%) of 75 serum samples were AIV seropositive, with 9.33% (7/75) in H5 AIV and 61.33% (46/75) in H9 AIV, and the seroprevalence of H5+H9 AIV was 6.67%. In addition, the result was negative in H3 or H7 AIV (Table 1). The seroprevalence ranged from 38.71% to 93.33% in different areas, the highest and lowest rates were recorded in Shenyang and Changchun (Table 1). In the 1<years <2 and > 5 year-old groups, the seroprevalence of the H5 AIV was 4% and 12% ($p>0.05$), and the seroprevalence of the H9 AIV was 24% and 80% ($p<0.01$), respectively (Fig. 1).

CDV exposure in captive tigers

The seroprevalence of CDV was 16% (12/75). Among the different areas, CDV seroprevalence varied from 0% (0/15) to 27.59% (8/29), and the highest rates were recorded in Harbin (Table 2). The CDV seroprevalence in the 1<year-old <2 and > 5 year-old group were 6% and 36% ($p<0.01$), respectively (Fig. 1). It was demonstrated that 3 (4%) of 75 serum samples were AIV+CDV seropositive, with 2.67% (2/75) in H9+AIV and 1.33% (1/75) in H5+H9+AIV (Table 2).

Discussion

Influenza infection in big cat species, especially in tigers, has been reported (Thiry et al. 2007, Wang et al. 2014). In this study, forty-eight (61.54%) of 75 serum samples were detected positive for AIV (H5 and H9) antibodies; this result indicates that tigers had been infected with H5 and H9 subtypes of AIV in the past, but not to H3 and H7. Although clinical symptoms were not observed in the AIV antibody positive tigers, there is a risk that tigers can be infected by the H5 and H9 subtypes of AIV, and serological tests of AIV may provide an early warning. Furthermore, the tigers involved in this study had not previously been vaccinated, and eating chicken from poultry markets is the most probable reason for the high seroprevalence in H5 and H9 AIV. The results are similar to those of a recent study (Imai et al. 2013). In addition, there are some migratory birds, including mallards, grebes and herons, that rest or breed in the same space as the captive *Siberian Tigers*. Therefore, migratory birds may accelerate the spread of influenza A viruses in tigers as previous reports (Webster et al. 1992). Moreover, AIV seroprevalences in tigers of different age groups were slightly different, the highest seroprevalence was the > 5 year-old group. This reveals that sub-adult tigers are more susceptible to AIV than adults. Many factors could be responsible for the variability in H5 and H9 AIV seroprevalence between the sub-adults and adults, and thus future studies are warranted.

CDV is a common infectious disease, causing death in domestic dogs and endangering wild carnivores (Deem et al. 2000). Serious CDV infections in seals, lions, tigers and leopards had been reported (Fujita et al. 2007). A previous study indicated that tiger CDV is an arctic-like strain similar to CDV in Baikal seals in Russia (Seimon et al. 2013). In this study, 12 (16%) serum samples were positive for the CDV antibody test, of which 4 (5.33%) were suspect, and 59 (78.67%) were negative. Comparing the presence of antibodies in the various age groups of the tigers, the seroprevalence in > 5 year-old was obviously higher than 1<years <2 group ($p<0.01$). The results are similar to the reports that there is a higher seroprevalence in older dogs (Acosta-Jamett et al. 2011). However, clinical symptoms were not observed in the CDV antibody positive tigers. These tigers may contribute to a continuous circulation of CDV within the environment, as has been reported in cats (Clegg et al. 2012).

Some studies indicate that H5, H9 and CDV can cause infection with clinical symptoms in tigers (Hu et al. 2016, Zhang et al. 2017); however, H5 and H9 can also cause asymptomatic infection in mammals (Fan et al. 2014). Considering AIV and CDV as a threat to tigers, serological tests of AIV and CDV were studied. The results indicate that tigers in this particular region in China are co-exposed to H5 and H9, H9 and CDV, and H5, H9 and CDV in captive *Siberian Tigers*. Previous research demonstrated that H7, H5 and H9 AIV co-infect herons in a city park in Jiangxi, China (Wang et al. 2014); however, there have been no studies regarding the co-infection of H5, H9 and CDV. Thus, particular attention should be paid to the co-exposure of H5, H9 and CDV, which may strengthen the pathogenicity of infectious diseases and result in the rapid decline of the captive tiger population.

To our knowledge, this is the first report of AIV (H5, H9) and CDV seroprevalence in captive *Siberian Tigers* in China. Serological tests of captive *Siberian Tigers* may provide an early warning of potentially novel AIV and CDV infections and is very important for the conservation of *Siberian Tigers*. The present study also provided baseline information for the timely execution of strategies and measures to control AIV (H5, H9) and CDV infection in captive *Siberian Tigers* and to assess resulting effects. Therefore, we should pay greater enhanced attention to this issue, and perform continuous serological surveillance. Therefore, it is necessary to implement integrated control and efficient management measures to prevent and control AIV and CDV infection in tigers.

Acknowledgements

This study was financially supported by the national key research and development program of China (2017YFD0501703, 2016YFD0501002) and the National Science and Technology Pillar Program during the Twelfth Five-year Plan Period (2013BAD12B04) and 973 Program (2013FY113600).

References

Acosta-Jamett G, Chalmers WSK, Cunningham AA, Cleaveland S, Handel IG, Bronsvoort BM (**2011**) Urban domestic dog populations as a source of canine distemper virus for wild carnivores in the Coquimbo region of Chile. Vet Microbiol 152: 247-257.

Clegg SR, Coyne KP, Dawson S, Spibey N, Gaskell RM, Radford AD (**2012**) Canine parvovirus in asymptomatic feline carriers. Vet Microbiol 157: 78-85.

Deem SL, Spelman LH, Yates RA, Montali RJ (**2000**) Canine distemper in terrestrial carnivores: a review. J Zoo Wildl Med 31: 441-451.

Fan S, Zhou L, Wu D, Gao X, Pei E, Wang T, Gao Y, Xia X (**2014**) A novel highly pathogenic H5N8 avian influenza virus isolated from a wild duck in China. Influenza Other Resp 8: 646-653.

Fujita K, Miura R, Yoneda M, Shimizu F, Sato H, Muto Y,

Endo Y, Tsukiyama-Kohara K, Kai C (**2007**) Host range and receptor utilization of canine distemper virus analyzed by recombinant viruses: Involvement of heparin-like molecule in CDV infection. Virology 359: 324-335.

Fukui D, Nagano M, Nakamura R, Bando G, Nakata S, Kosuge M, Sakamoto H, Matsui M, Yanagawa Y, Takahashi Y (**2013**) The Effects of Frequent Electroejaculation on the Semen Characteristics of a Captive Siberian Tiger (Panthera tigris altaica). J Reprod Dev 59: 491-495.

He S, Shi J, Qi X, Huang G, Chen H, Lu C (**2015**) Lethal infection by a novel reassortant H5N1 avian influenza A virus in a zoo-housed tiger. Microbes Infect 17: 54-61.

Hu T, Zhao H, Zhang Y, Zhang W, Kong Q, Zhang Z, Cui Q, Qiu W, Deng B, Fan Q, Zhang F (**2016**). Fatal influenza A (H5N1) virus infection in zoo-housed Tigers in Yunnan Province, China. Sci Rep 6: 25845.

Imai M, Herfst S, Sorrell EM, Schrauwen EJ, Linster M, De Graaf M, Fouchier RA, Kawaoka Y (**2013**) Transmission of influenza A/H5N1 viruses in mammals. Virus Res 178: 15-20.

Kimber KR, Kollias GV, Dubovi EJ (**2000**) Serologic survey of selected viral agents in recently captured wild North American river otters (Lontra canadensis). J Zoo Wildl Med 31: 168-175.

Mushtaq MH, Juan H, Jiang P, Li Y, Li T, Du Y, Mukhtar MM (**2008**) Complete genome analysis of a highly pathogenic H5N1 influenza A virus isolated from a tiger in China. Arch Virol 153: 1569-1574.

Myers DL, Zurbriggen A, Lutz H, Pospischil A (**1997**) Distemper: not a new disease in lions and tigers. Clin. Diagn. Lab. Immunol 4: 180-184.

Rastogi A, Hickey GM, Badola R, Hussain SA (**2014**) Understanding the local socio-political processes affecting conservation management outcomes in Corbett Tiger Reserve, India. Environ Manage 53: 913-929.

Seimon TA, Miquelle DG, Chang TY, Newton AL, Korotkova I, Ivanchuk G, Lyubchenko E, Tupikov A, Slabe E, McAloose D (**2013**) Canine distemper virus: an emerging disease in wild endangered Amur tigers (Panthera tigris altaica). mBio 4: e00410-13.

Thiry E, Zicola A, Addie D, Egberink H, Hartmann K, Lutz H, Poulet H, Horzinek MC (**2007**). Highly pathogenic avian influenza H5N1 virus in cats and Other carnivores. Vet Microbiol 122: 25-31.

Wang G, Zhang T, Li X, Jiang Z, Jiang Q, Chen Q, Tu X, Chen Z, Chang J, Li L, Xu B (**2014**) Serological evidence of H7, H5 and H9 avian influenza virus co-infection among herons in a city park in Jiangxi, China. Sci Rep 4: 6345.

Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y (**1992**) Evolution and ecology of influenza A viruses. Microbiol. 56: 152-179.

Xia X, Gao Y, Hu R, Wang L, Liu D, Zou X, Huang G, He W, Wang W, Su W, Liu W (**2003**) The first finding of tiger influenza by virus isolation and specific gene amplification. Chinese Vet Sci 23: 107-110.

Yu Z, Sun W, Li X, Chen Q, Chai H, Gao X, Guo J, Zhang K, Wang T, Feng N, Zheng X, Wang H, Zhao Y, Qin C, Huang G, Yang S, Hua Y, Zhang X, Gao Y, Xia X (**2015**) Adaptive amino acid substitutions enhance the virulence of a reassortant H7N1 avian influenza virus isolated from wild waterfowl in mice. Virology 476: 233-239.

Zhang H, Shan F, Zhou X, Li B, Zhai J, Zou S, Wu M, Chen W, Zhai S, Luo M (**2017**) Outbreak and genotyping of canine distemper virus in captive Siberian tigers and red pandas. Sci Rep 7: 8132.