Chinese Mitten Crabs (*Eriocheir Sinensis*) Could Act As Vehicles of Spreading Avian Influenza Virus

Weiwei Ma
Shenyang Agriculture University
Chenyang Ren
Shenyang Agricultural University
Qingbiao Hu
Shenyang Agriculture University
Xiaodong Li
Shenyang Agriculture University
Yali Feng
Shenyang Agriculture University
Ying Zhang (yingzhang18@syau.edu.cn)
Shenyang Agriculture University https://orcid.org/0000-0002-3476-6237

Short report

Keywords: Avian influenza virus, Chinese mitten crab, aquatic, spread

Posted Date: November 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1007016/v1

Abstract

Avian influenza virus (AIV) possessed significant risk to various animals and human health. Wild birds, especially waterfowls are considered to be the natural reservoir of AIVs. The ecology of AIV is still far from being fully understood. Chinese mitten crabs are nonnegligible biotic factor in AIV ecosystem. We analyzed the ability of Chinese mitten crabs accumulate and spread AIV. We found that AIV remain infectious in water only for 36 hours but persist in crabs for 48 hours. Crabs grills and gastrointestinal tracts accumulated AIV with higher titers than viral water. Crabs could accumulate AIV from contaminated water, carry the virus and spread to naïve crabs via surrounding water. Our study identified Chinese mitten crab as a novel transmission vehicle in AIV ecosystem.

1 Introduction

Avian Influenza virus (AIV) is important pathogen for both human and animals. AIV is a RNA virus composed of 8 genome segments. Hemagglutinin (HA) and neuraminidase (NA) are two major glycoproteins on the surface of AIV. AIV can be classified into 18 HA (H1-H18) and 11NA (N1-N11) subtypes based on the different antigenicity of HA and NA. All of these subtypes have been found in wild birds excepting for H17N10 and H18N11 subtypes [1, 2]. So it has been widely accepted that wild birds, especially waterfowls are the natural reservoir of AIVs. Occasionally, AIV in waterfowls could spill over to domestic poultry, livestock, marine mammals and even humans [3, 4].

AIVs mainly replicate in the intestine tract cells of waterfowls [5], so the infected bird faeces may contain high concentration of AIVs. The faecal-oral route is considered to be the primary AIV transmission mode in waterfowls. It has been reported that AIVs can remain infectious in aquatic environment for more than seven months [6]. As a result, it makes the aquatic environment become an epidemic focus where AIV could transmit among waterfowl and other animal living in the same area [7].

Chinese mitten crab (*Eriocheir sinensis*) belongs to *Malacostraca*, *Decapoda*, *Grapsidae*. It is an omnivorous animal which mainly feed on plant and animal detritus [8]. In the wild, the crabs could also be the prey of waterfowl and poultry. The farming industry of Chinese mitten crabs involves 30 provinces in China, which has become the largest industry of single species of freshwater fishery [9]. Chinese mitten crabs are widely existing in freshwater lakes, rivers and brackish waters in China [10]. Sharing same aquatic habitats and being in predation relation with waterfowls, Chinese mitten crabs might become a transmission biotic factor in AIV ecosystem. In this study we evaluated the function of Chinese mitten crab in AIV ecosystem.

2 Materials And Methods

2.1 Chinese mitten crabs

The Chinese mitten crabs which weighed 10.0 ± 1.0 g were giving by Panjin Guanghe Crab industry Co Ltd. The crabs were kept in aerated water for 2 weeks at 18 ℃ ahead. Aerated water was made by pumping air into 20 Liter tap water with an air pump (20L/min) overnight. Finally, the dissolved oxygen and pH level of the aerated water was 6.4mg/ml and 7.2 respectively.

2.2 Virus and cell

H9N2 avian influenza virus A/chicken/Liaoning/07/2016 was isolated from chicken during routine surveillance. Virus stock was prepared by inoculation of Madin-Darby canine kidney (MDCK) cells. The viral titer was determined by 50 % tissue culture infectious dose ($TCID_{50}$).

2.3 Persistence of Avian influenza virus in the aerated water

The viral water was made by adding $10^{7.5}$ $TCID_{50}$ AIV into 1 Liter aerated water, mixing thoroughly. 3 tanks with 1L viral water were put into a biosafety cabinet at 18 ℃. 1 ml water sample was taken from each tank after 0, 1, 3, 8, 12, 24, 36, 48 and 60 hours(h) respectively to test the viral titer in MDCK cells. The limitation of virus detection was set as 0.5 $TCID_{50}$/ml.

2.4 AIV accumulation in Chinese mitten crabs

5 group of 3 crabs were distributed into 5 viral water tanks. At 0, 1, 3, 8 and 12h post incubation (hpi), 1 group of 3 crabs were rinsed and euthanized. Crabs gills, hepatopanpancreas, gastrointestinals, muscles and viral water sample were collected for viral titration.

2.5 AIV accumulating limitation of crabs

6 groups of 3 crabs were incubated in viral water. Every 12h, the tank water was changed with fresh viral water until 60 hours later. Since 0h after incubation, 1 group of crabs were rinsed and euthanized for gills collection before water changing. The viral water sample was collected at the same time.

2.6 AIV spreading activity of Chinese mitten crabs

Groups of 3 crabs were incubated in viral water for 8h as inoculated groups. After rinsing thoroughly, the inoculated crabs were transferred into fresh water. Groups of 3 naïve crabs, as sentinel groups, were put into each tank. During the first co-tanking 4h, every 0.5h 1 group of inoculated and sentinel crabs were rinsed and euthanized. Their grills and water sample were collected at the same time for viral titration.

3 Results

3.1 AIV persisted for a shorter time in lab condition

As shown in figure 1, AIV could maintain similar infectivity for 3h. Viral titer began to drop at 8hpi. At 24hpi, viral titer dropped by half. There's no detectable virus in water after 36h.

3.2 Chinese mitten crabs accumulated and preserved AIV longer than in water

Groups of 3 crabs were inoculated by incubating in viral water. As shown in Table 1, AIV could be detected in crabs' grills and intestinal tracts since 1hpi. The crabs' grills accumulated AIV more efficiently than their gastrointestinal tracts. The grills viral titers were higher than viral water since 8hpi. At 36hpi, the crab viral titers began to drop. Whereas, water viral titer kept on dropping from the beginning of the experiment. AIV could still be detected in 1 crab's grill at 36hpi and in 1crab's grill and gastrointestinal tract at 48hpi. No virus was detected in other organs of the crabs. It's indicated that AIV could "infect" crabs through their respiratory and digestive systems. There's no detectable AIV after 48hpi in neither crabs nor in water.

Table 1
AIV accumulation in Chinese mitten crabs

Hours post inoculation (hpi)	Viral titer ($log_{10}TCID_{50}/mL$)[a]				
	Gill	Gastrointestinal tract	Hepatopancreas	Muscle	Water
0	-	-	-	-	4.25
1	2.62/2.02/2.01	2.82/2.62/-	-	-	3.75
3	3.14/3.07/2.83	3.11/-/-	-	-	3.50
8	4.96/4.91/4.61	2.92/-/-	-	-	2.75
12	4.88/4.45/4.18	2.18/-/-	-	-	3.25
24	4.97/3.94/-	-	-	-	2.50
36	2.68/-/-	-	-	-	2.25
48	2.70/-/-	2.62/-/-	-	-	1.50
60	-	-	-	-	-

[a]5 group of 3 crabs were distributed into 5 viral water tanks. After 0, 1, 3, 8 and 12h incubation, 1 group of 3 crabs were rinsed and euthanized. Crabs organs and viral water were collected for viral titration in MDCK cells. -, no virus was detected in samples.

3.3 Chinese mitten crabs accumulated AIV with a titer but not time limitation

The crabs surrounding viral water was refreshed every 12h. The crab viral titers were consistent but higher than viral water. So the Chinese mitten crabs could continue accumulating AIV but might be confined by their size or water viral titer.

3.4 Chinese mitten crabs spread AIV to surrounding water and naïve crabs

We inoculated crabs by incubating them in viral water for 8h and used them as the inoculated groups. Groups of 3 naïve crabs were treated as sentinel groups. As shown in Table2, AIV could be detected from inoculated crabs until 8hpi. Since 1hpi, AIV was detected from 2 of the 3 sentinel crabs. At 2.5hpi, AIV was only detected from 1 sentinel crab. There's no detectable AIV in sentinel groups after 3hpi. AIV was detected from water at 1 and 1.5hpi. No alive virus in water after 2h. The inoculated groups contained higher viral titer than sentinel groups and water. The result indicated that, AIV contaminated Chinese mitten crab could spread virus into water and "infect" the naïve crabs.

Table 2
AIV spreading activity of Chinese mitten crabs.

Hours post inoculation (hpi)	Viral titer ($log_{10}TCID_{50}$/mL)[a]		
	Inoculated crabs	Sentinel crabs	Water
0	4.96/4.91/4.61	-	-
0.5	4.05/3.71/3.67	-	-
1	3.74/3.62/3.18	2.89/2.34/-	2.50
1.5	3.84/2.85/2.35	2.95/2.45/-	2.25
2	3.68/3.17/2.13	2.60/2.35/-	-
2.5	3.24/2.68/2.16	2.68/-/-	-
3	2.49/2.32/-	-	-
3.5	2.70/-/-	-	-
4	2.57/-/-	-	-
8	2.49/-/-	-	-
12 to 60*	-	-	-

[a]Groups of 3 crabs were inoculated by incubating in viral water for 8h. After rinsing thoroughly, the inoculated crabs were transferred into fresh water tanks. Groups of 3 sentinel crabs were put into each tank. At designated time points, 1 group of inoculated and sentinel crabs were rinsed and euthanized. Their grills and water were collected at the same time for viral titration in MDCK cells. -, no virus was detected in samples. *, crabs and water samples were collected every 12h.

4 Discussion

Pathogenic ecology of AIV including host, pathogen and environment. The biotic and abiotic factors of environment play important roles in AIV spreading process. Waterfowls especially the migratory waterfowls can disseminate AIV along their flyways. There's high possibility that Chinese mitten crabs could be contaminated by AIV shedding from the waterfowls.

Researchers found AIV RNA in small aquatic vertebrates and invertebrates, such as water fleas [11], bamboo shrimp (*Atyopsis moluccensis*), clams (*Corbicula fluminea*), freshwater snails (*Physa spp.*), zebra mussels (*Dreissena ploymorpha*), crayfish and Mediterranean cone shell (Conus sp.). Experiments indicated these aquatic animals can accumulate AIVs through water filtering but the infectivity of these accumulated AIV had not been evaluated yet [12–17]. Our study for the first time evaluated the infectivity of accumulated AIV in crabs. Furtherly, we determined the AIV spreading activity of Chinese mitten crabs. Further study should be conducted to evaluate the AIV transmission between Chinese mitten crabs and their predators.

5 Conclusion

In the present study, we evaluated the Chinese mitten crab AIV accumulating and spreading activity. AIV could be accumulated in multiple organs of the crabs and stay infectious longer than in water. Most importantly, AIV could be carried by the crabs into freshwater and transmitted to the naïve crabs. Our study indicates that Chinese mitten crabs is an important factor in the AIV ecosystem.

List Of Abbreviations

AIV: avian influenza virus; HA: hemagglutinin; NA: neuraminidase; MDCK: Madin-Darby canine kidney; $TCID_{50}$: 50% tissue culture infectious dose; h: hours; hpi: hours post inoculation

Declarations

Ethics approval and consent to participate

The animal research was approved by the Committee on the Ethics of Animal Experiments of Key Laboratory of Livestock Infectious Diseases in Northeast China, Ministry of Education.

Consent for publication

All authors consent to the publication of the manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (32170539, 32000357), “Xingliao Talent Plan” of Liaoning (XLYC2007114), Key Research and Development Program of Liaoning

(2020JH2/10200035)

Author contributions

Y.Z. designed the experiment. W.M., C.R., Q.H., Y.F., conducted the experiments. W.M., Q.H., X.L. and Y.Z. analyzed the data. W.M. and Y.Z. wrote the manuscript.

All data generated or analysed during this study are included in this published article

Acknowledgements

Not applicable.

References

1. Tong S, Li Y, Rivailler P, Conrardy C, Castillo DA, Chen LM, et al. A distinct lineage of influenza A virus from bats. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(11):4269-74. https://doi.org/10.1073/pnas.1116200109
2. Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, et al. New world bats harbor diverse influenza A viruses. PLoS Pathog. 2013;9(10):e1003657. https://doi.org/10.1371/journal.ppat.1003657
3. Li Y, Shi J, Zhong G, Deng G, Tian G, Ge J, et al. Continued evolution of H5N1 influenza viruses in wild birds, domestic poultry, and humans in China from 2004 to 2009. J Virol. 2010;84(17):8389-97. https://doi.org/10.1128/JVI.00413-10
4. Chen H, Li Y, Li Z, Shi J, Shinya K, Deng G, et al. Properties and dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in western China. J Virol. 2006;80(12):5976-83. https://doi.org/10.1128/JVI.00110-06
5. Webster RG, Yakhno M, Hinshaw VS, Bean WJ, Murti KG. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology. 1978;84(2):268-78. https://doi.org/10.1016/0042-6822(78)90247-7
6. Ramey AM, Reeves AB, Drexler JZ, Ackerman JT, De La Cruz S, Lang AS, et al. Influenza A viruses remain infectious for more than seven months in northern wetlands of North America. Proceedings Biological sciences. 2020;287(1934):20201680. https://doi.org/10.1098/rspb.2020.1680
7. Ito T, Okazaki K, Kawaoka Y, Takada A, Webster RG, Kida H. Perpetuation of influenza A viruses in Alaskan waterfowl reservoirs. Arch Virol. 1995;140(7):1163-72. https://doi.org/10.1007/BF01322743
8. Zhang L, Zhu C, Yang J, Song W, Jiang Z. Environmental Investigation of Crab Breeding Farm in Yangtze River Estuary. Fisheries Science and Technology Information (In Chinese). 1988(01):3-13.
9. Sui L, Zhang F, Wang X, Bossier P, Sorgeloos P, Hänfling B. Genetic diversity and population structure of the Chinese mitten crab Eriocheir sinensis in its native range. Marine Biology. 2009;156(8):1573-83. https://doi.org/10.1111/j.1365-294X.2008.03850.x

10. Zhang D, Qi T, Liu J, Liu Q, Jiang S, Zhang H, et al. Adaptively differential expression analysis in gill of Chinese mitten crabs (Eriocheir japonica sinensis) associated with salinity changes. International journal of biological macromolecules. 2018;120(Pt B):2242-6. https://doi.org/10.1016/j.ijbiomac.2018.08.054
11. Abbas MD, Nazir J, Stumpf P, Marschang RE. Role of water fleas (Daphnia magna) in the accumulation of avian influenza viruses from the surrounding water. Intervirology. 2012;55(5):365-71. https://doi.org/10.1159/000334691
12. Pathak AP, Murugkar HV, Nagarajan S, Sood R, Tosh C, Kumar M, et al. Survivability of low pathogenic (H9N2) avian influenza virus in water in the presence of Atyopsis moluccensis (Bamboo shrimp). Zoonoses Public Health. 2018;65(1):e124-e9. https://doi.org/10.1111/zph.12420
13. Faust C, Stallknecht D, Swayne D, Brown J. Filter-feeding bivalves can remove avian influenza viruses from water and reduce infectivity. Proceedings Biological sciences. 2009;276(1673):3727-35. https://doi.org/10.1098/rspb.2009.0572
14. Huyvaert KP, Carlson JS, Bentler KT, Cobble KR, Nolte DL, Franklin AB. Freshwater clams as bioconcentrators of avian influenza virus in water. Vector borne and zoonotic diseases (Larchmont, NY). 2012;12(10):904-6. https://doi.org/10.1089/vbz.2012.0993
15. Oesterle PT, Huyvaert KP, Orahood D, Mooers N, Sullivan H, Franklin AB, et al. Failure of transmission of low-pathogenic avian influenza virus between Mallards and freshwater snails: an experimental evaluation. J Wildl Dis. 2013;49(4):911-9. https://doi.org/10.7589/2012-04-111
16. Stumpf P, Failing K, Papp T, Nazir J, Böhm R, Marschang RE. Accumulation of a low pathogenic avian influenza virus in zebra mussels (Dreissena polymorpha). Avian Dis. 2010;54(4):1183-90. https://doi.org/10.1637/9162-111709-Reg.1
17. Root JJ, Ellis JW, Shriner SA. Effects of freshwater crayfish on influenza A virus persistence in water. Zoonoses Public Health. 2020;67(3):300-7. https://doi.org/10.1111/zph.12688

Figures

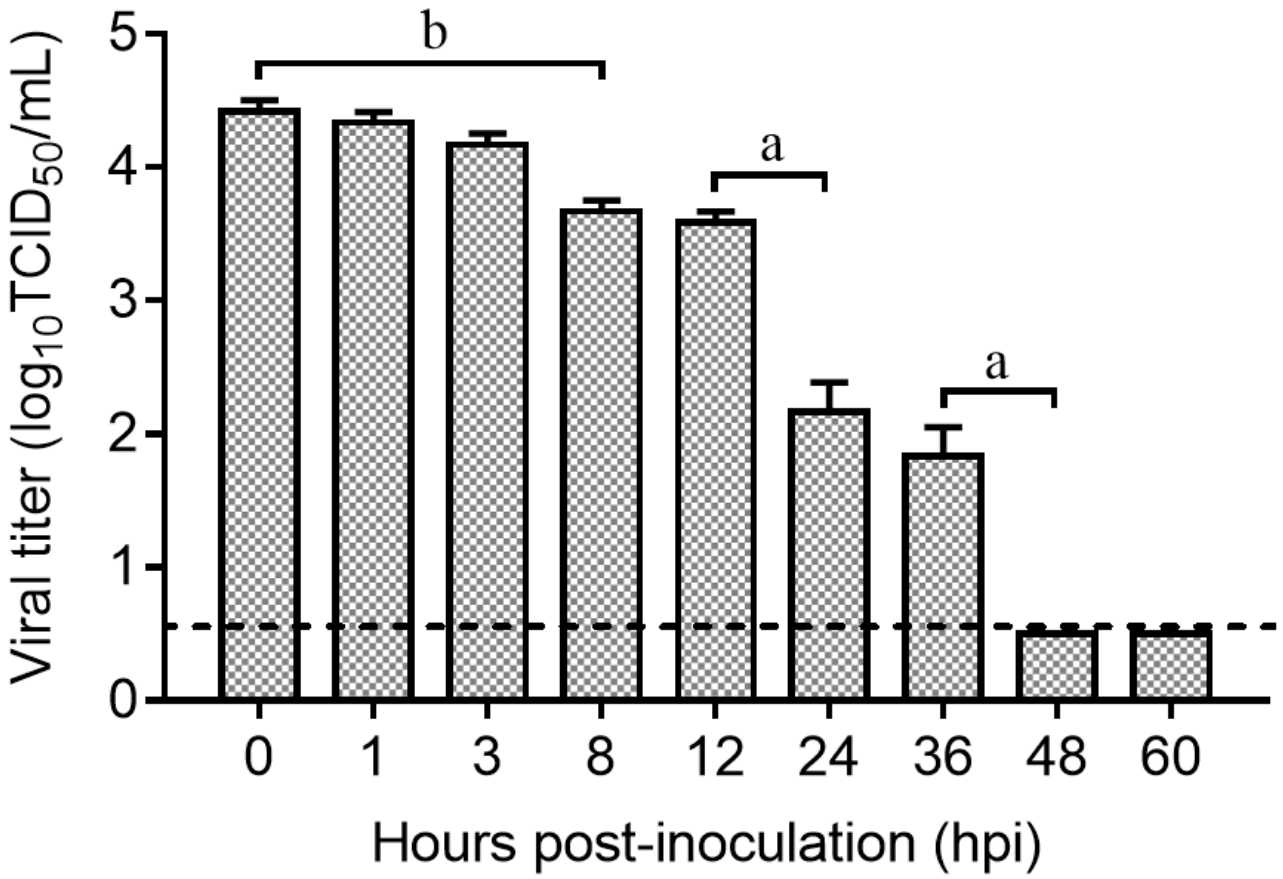

Figure 1

Persistence of AIV in aerated water. 107.5TCID50 AIV virus was added to 1L aerated water at 18°C. Water samples were collected at designated time points and titrated on MDCK cells. The data shown are the means of three replicates; the error bars indicate standard deviations. The dashed lines indicate the lower limit of virus detection. Data were analyzed using analysis of variance (ANOVA) in GraphPad Prism version 9.0 (GraphPad Software Inc., CA, USA). Significance was analyzed by using a one-way ANOVA with post-hoc tests. a, $P<0.001$; b, $P<0.05$.

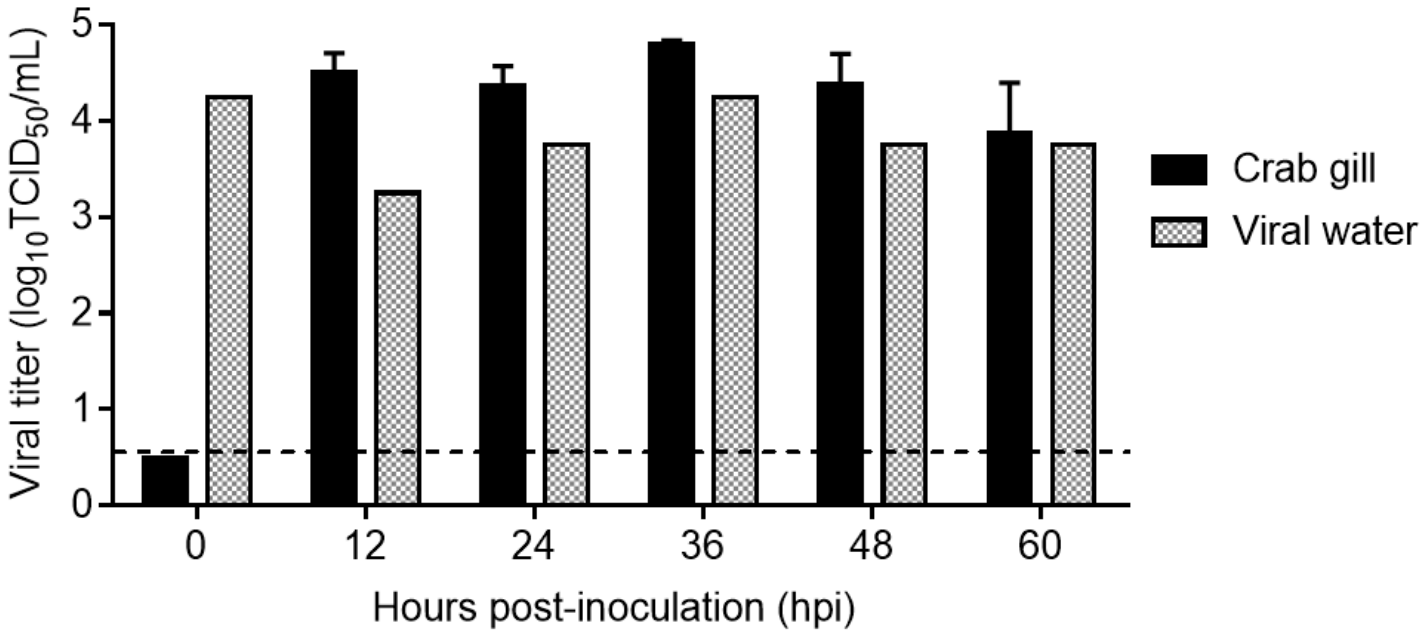

Figure 2

AIV accumulating limitation of crabs. 6 groups of 3 crabs were transferred into 1L viral water. Every 12h, the tank water was changed with fresh viral water. 1 group of crabs were rinsed and euthanized for gills collection at designated time points. The viral water sample was collected at the same time. Crabs and water samples were titrated in MDCK cells. The data shown are the means of three replicates; the error bars indicate standard deviations. The dashed lines indicate the lower limit of virus detection.