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Title of Article: Current status of viral disease spread in Korean horn beetle, *Allomyrina dichotoma* (Coleoptera: Scarabeidae)

Seokhyun Lee, Hong-Geun Kim, Kwan-ho Park, Sung-hee Nam, Kyu-won Kwak and Ji-young Choi*

Industrial Insect Division, National Institute of Agricultural Science, Rural Development Administration, Jeollabuk-do, Korea

Abstract

The current market size of insect industry in Korea is estimated at 300 million dollars and more than 500 local farms are related to many insect industry. One of the strong candidates for insect industry is Korean horn beetle, *Allomyrina dichotoma*. Early this year, we reported a viral disease extremely fatal to *A. dichotoma* larvae. While we were proceeding a nationwide investigation of this disease, it was informed that similar disease symptom has been occurred occasionally during past over 10 years. The symptom can be easily confused with early stage of bacterial infection or physiological damage such as low temperature and high humidity. A peroral infection with the purified virus to healthy larvae produced a result that only 21% of larvae survived and became pupae. Although some of the survived adult beetle was deformational, many of them had no abnormal appearance and even succeeded in mating. Later, these beetles were examined if they were carrying the virus, and all except one were confirmed as live virus carrier. This implies that these beetles may fly out and spread the disease to the nature. We found the evidence for this possibility by collecting a few wild *A. dichotoma* larvae which were virus infected, near two local farms rearing *A. dichotoma* larvae. So far, transovarial transmission of this virus to the eggs, or horizontal transmission to other commercially reared insects is not known yet.

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Introduction

Historically in agriculture, most insects have been considered as pests causing damages to agricultural economy, but that point of view is being changed. Nowadays, insects are considered as valuable resources in many aspects including viable human diet and animal feed resources, pharmaceutical and medicinal product, pollinator insect, natural enemy of insect pest, and environmental cleanup. Especially, many insects belong to *Coleoptera*, *Orthoptera*, *Hymenoptera*, and *Lepidoptera* have been consumed as human diet all over the world. Comparing to other livestock, these insects have many benefits such as good protein source or high reproduction rate. It has been reported that insects have nutritional value, including high protein, unsaturated fatty acids, carbohydrates, vitamins, and minerals (Raubenheimer and Rothman, 2013; Youm *et al*., 2012). Also, rearing insects takes relatively low cost and they can be reared in large scale with easy management. For these reasons, they are getting the limelight as alternative food source for future (Van Huis, 2013). In Korea, the current market size of the insect industry is

***Corresponding author.**
Ji-young Choi
Industrial Insect Division, National Institute of Agricultural Science(NAS), RDA
Tel: +82-63-238-2992 / FAX: +82-63-238-3833
E-mail: choijy7@korea.kr

estimated at 300 million dollars and more than 500 local farms are related to many insect industry. One of the strong candidates for insect industry is Korean horn beetle, *Allomyrina dichotoma*. The adult beetle is very popular as pet and educational purpose, while the larvae have been consumed as an ingredient of oriental medicine in Korea for a long time. They have been used for the treatment of anti-obesity and diabetes (Sagisaka *et al.,* 2001), and it has been reported that *A. dichotoma* has many biologic effects including anti-bacterial, (Koyama *et al*., 2006), anti-hepatotoxic (Lee and Lee, 2009), anticytotoxic (Kim *et al*., 2007), anti-oxidant (Suh *et al*., 2010) effects.

The common diseases occurring in Korean horn beetle are white muscardine and green muscardine disease when infected by fungi *Metarhizium anisopliae* and *Beauveria bassiana*, and bacterial diseases caused by *Bacillus thuringiensis* and *Serratia marcescens*. It is also widely known that the beetles are suffered by microsporidia and parasitic mite. Generally, these diseases occur in dense and large scaled larvae-rearing ground and in most case, the disease can be prevented or controlled by improving the insanitary condition including incompletely fermented fodder, too high humidity, and poor ventilation. Recently, we reported that a fatal viral disease is diagnosed in *A. dichotoma* larvae reared in local farms in Korea (Lee *et al*., 2015). The virus was identified as *Oryctes rhinoceros nudivirus* (*OrNV*) which is known to cause severe disease in coconut palm rhinoceros beetle, *Oryctes rhinoceros*, in Southeastern Asia, and there, it is used as biological control agent to reduce the pest population for coconut palm oil industry (Huger, 2005; Ramle *et al*., 2005). *OrNV* is a double strand DNA virus having an enveloped rod-shaped virion, and multiplies in the midgut and fat body of infected larvae (Wang *et al*., 2011; Huger, 2005). The virus is released from the infected larvae on the breeding site and infects other larvae. While we investigate this disease, several farmers answered that similar disease symptom had been occurred occasionally during last 10 years. The symptom can be easily confused with early stage of bacterial infection or physiological damage including temperature and moisture. Therefore, this implies that it is possible that the disease might have spread among Korean horn beetle over 10 years nationwide, but just was not officially reported until 2012, when a local farm in Cheongwon-gun reported a massive death of *A. dichotoma* larvae. On this investigation, we found that this viral disease already spread all over Korea and became a devastating threat for Korean farmers and insect industry.

Materials and Methods

Collection of diseased larvae

The diseased larvae were collected from several local farms rearing larvae throughout Korea from Apr. 2014 to Sep. 2015. Collection sites include Anseong-si, Buyeo-gun, Cheongwon-gun, Daejeon-si, Geoje-si, Geumsan-gun, Gyeongsan-si, Hoengseong-gun, Muju-gun, Naju-si, Namyangju-si, Pocheon-si, Siheung-si, Uiseong-gun, Wanju-gun, Yeongam-gun, Yongin-si, and Youngdong-gun.

Diagnosis of virus infection

To diagnose the diseased larvae, DNA was isolated from diseased larvae and viral DNA was amplified by PCR with three pairs of primers (primer AdV-F1 and -R1, primer AdV-F2 and-R2, and primer AdV-F3 and –R3) which were previously designed in our lab (Lee *et al.*, 2015). First, the hemolymph was extracted by cutting a leg of a diseased larva, and then it was centrifuged 2,000 x g for 15min at 4°C to remove cell debris. DNA was extracted with Wizard plus SV miniprep kit (Promega, Madison, WI) as instructed by the manufacturer. PCR diagnosis of viral DNA was performed under following condition: a denaturation at 95°C for 3 min, 35 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 10 min. All three pairs of primers were used under the same condition. For amplification, AccuPower PCR premix (BioNeer, Seoul, Korea) was used as instructed.

Peroral infection of OrNV

Peroral virus infection of third instar larva was accomplished by dropping 30 μL of hemolymph extracted from diseased larvae on the mouthparts of thirty healthy larvae, respectively. The infected larva was independently reared in plastic container filled with moist fermented oak sawdust. To avoid other infection, the sawdust was sterilized before use. The infection experiment was repeated for three times.

Results and Discussion

Nationwide State of Viral disease

The diseased larvae of Korean horn beetle were collected from local farms rearing larvae throughout Korea. Table 1 describes the

Table 1. Current state of *OrNV* disease spread to *A. dichotoma* larvae rearing farms throughout Korea.

Location		Collection date	*OrNV* positive / examined larvae
Gyeonggi do	Pocheon-si	2014. 5.	8/8
		2015. 6.	20/20
	Siheung-si	2014. 5.	0/10
		2015. 2.	14/16
		2015. 7.	16/16
	Namyangju-si	2014. 7.	4/8
		2015. 7.	26/26
	Anseong-si	2014. 6.	4/6
	Yongin-si	2015. 7.	6/6
Gangwon-do	Hoengseong-gun	2015. 6	0/10
Chungcheongbuk-do	Youngdong-gun	2014. 4.	12/12
		2014.11.	20/20
		2015. 6	9/9
	Cheongwon-gun	2014. 4.	4/4
Chungcheongnam-do	Daejeon-si	2014. 6.	13/13
		2015. 6.	48/48
		2015. 6. (wild larvae)	1/2
	Geumsan-gun	2015. 6.	21/21
	Buyeo-gun	2015. 6.	2/2
Gyeongsangbuk-do	Uiseong-gun	2014.12.	30/30
	Gyeongsan-si	2014.12.	2/2
		2015. 7.	47/47
Gyeongsangnam-do	Geoje-si	2015. 6.	17/17
		2015. 6. (wild laevae)	2/4
Jeollabuk-do	Muju-gun	2015. 9.	8/9
	Wanju-gun	2014.12.	41/41
		2015. 4.	15/15
Jeollanam-do	Naju-si	2015. 5.	20/20
	Yeongam-gun	2015. 5.	18/18

collection date, location, and sample numbers, and the location map is shown in Fig. 1. In several locations, samples were collected couple of times in the space of several months to a year. It was because in some locations, the viral disease was not found or not severe, and in a few farms, the disease was examined again after

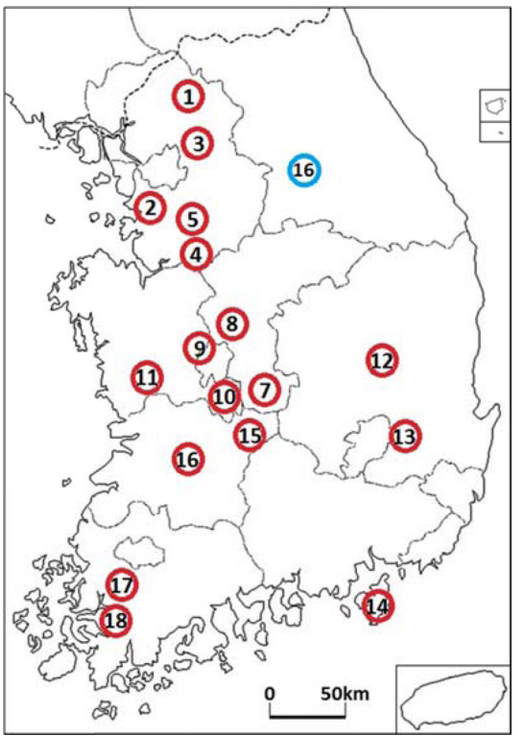

Fig. 1. Location map of *OrNV* disease-diagnosed farms. Red circle is where collected larvae were diagnosed as OrNV positive, and larvae from blue circle,*OrNV* was not detected. Each number refers to following province: 1: Pocheon-si, 2: Siheung-si, 3: Namyangju-si, 4: Anseong-si, 5: Yongin-si, 6: Hoengseong-gun, 7: Youngdong-gun, 8: Cheongwon-gun, 9: Daejeon-si, 10: Geumsan-gun, 11: Buyeo-gun, 12: Uiseong-gun, 13: Gyeongsan-si, 14: Geoje-si, 15: Muju-gun, 16: Wanju-gun, 17: Naju-si, 18: Yeongam-gun.

the farmer tried to improve the rearing conditions. Result is, the severity of disease did not ease but even became worse. Currently, the only clean area is Gangwon-do, as shown in Fig. 1. However, it could be simply because there are not many larvae-rearing farms in Gangwon-do, and they are relatively small scaled. Thus, they hardly needed to trade their larvae for crossbreeding with the farmers from other province, and kept their beetle clean from the viral disease. However, this luck did not visit other farms. Many farmers trade their larvae with others for many reasons and this accelerates the fast and wide spread of the disease all over the country. Moreover, many local farms are located near mountains or oak forest filled with wild beetles. The crossbreeding between wild and reared beetles is happening already in several farms, and this increases the possibility of virus transmission to the wild *A. dichotoma*. The evidence for this possibility was found in Daejeon-si and Geoje-si. As described in table 1, we collected a few wild larvae around a local farm, and they were diagnosed as *OrNV* positive.

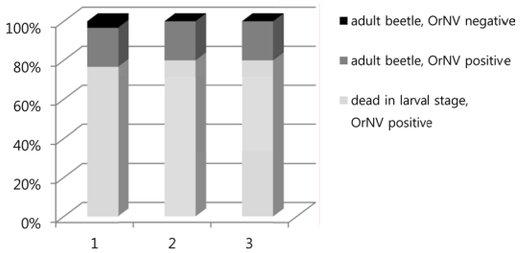

Fig. 2. Mortality and metamorphosis ratio after peroral infection. Thirty healthy larvae were infected with 30 μL of hemolymph from diseased larvae, orally, and only 21.1% of larvae went through metamorphosis and became adult beetle. Among the survived adult beetle, 95% of them were diagnosed as *OrNV* positive. Peroral infection experiment was repeated for three times.

Progression and Transmission of Disease

The visible symptom development of the viral disease was appeared after one week post infection, and the diseased larvae began to be killed after two to three weeks. Hemolymph was taken from every dead larva, and it was examined by PCR for viral DNA amplification with primers AdV-F1/R1, -F2/R2, -F3/R3. It was confirmed that all the perished larvae were infected by *OrNV*. Finally, only 21.1% of larvae went through metamorphosis and became adult beetle as shown in Fig. 2. Among them, only 58% of survived adult beetle could succeed in mating, while the others were deformational and died soon. After these adult beetles were dead, the midgut of those defected adult beetles was homogenized in distilled water, and DNA was isolated to examine for *OrNV* infection by PCR. All except one beetle were *OrNV* positive. This implies that infected but survived beetle may act as very active "flying" virus carrier.

Among the survived adult beetles, nine of them were successfully mated with healthy beetles and laid eggs. Six of them were female and three were male. When these adults died after they laid eggs, they were also examined for *OrNV* infection by PCR and resulted in all *OrNV* positive. To find out the possibility of transovarial transmission of the virus, three eggs from every nest were taken and examined by PCR. No viral DNA was amplified from the eggs. However, after the larvae hatched out, some of the larvae were diagnosed as *OrNV*-infected and were perished very early. This should be due to the hatched young larvae had the feeding activity from the same ground that the infected female had feeding and excretory activity. Therefore in nature, too, the infected adult beetle may spread this virus to the offspring in indirect manner.

Also, peroral infection of *Protaetia brevitarsis* (Coleoptera: Cetoniidae) with *OrNV* was executed. Because *A. dichotoma* and *P. brevitarsis* share the same food source, oak sawdust and have been used in traditional medicine for the same treatment, we tried to find out whether this viral disease can affect *P. brevitarsis*. However, even though both beetles can be infected by other pathogens including fungi *M. anisopliae* and *B. bassiana*, and bacteria *B. thuringiensis* and *S. marcescens*, in the experiment more than five times repeated with virus, none of *P. brevitarsis* was diagnosed as *OrNV* positive.

Sometimes the farmers may not realize exactly that they have disease issue in their farms. Because most local farms are growing the larvae in the open field, covered with plastic greenhouse and stuffed with oak sawdust in the ground, it is not easy to find the disease spreads in the sawdust pile. The diseased larvae will be rotted away and only the larvae near the dead larva will be infected. Unless the disease is extremely severe in the farm, the disease will not affect the whole bunch of larvae during the spring and early summer. However, the viral disease cause devastating loss especially when the larvae are harvested and stored for overwintering or waiting to be sold to a merchandiser. During the storage period, one or two hundreds of larvae are stored together in a big plastic container filled with moist sawdust for couple of months at low temperature. Thus, a few virus infected larvae can easily transmit the virus and kill the whole box of larvae. The transmission of virus is mostly made by having the feeding and excretory activity in the same fodder, and also by the cannibal behavior of larvae. The motion activity of a diseased larva is greatly reduced and these "weak" larvae or diseased cadaver get easily eaten by other larvae. In a severely diseased farm, more than 70% of container filled with larvae can be annihilated. Therefore, early detection and removal of the diseased larvae from the container box are extremely important. To analyze the virus, full genome sequencing is under way and it will be compared with *OrNV* genome originated from Malaysia. Also, although we assume that the virus has landed on Korea for longer than ten years ago, the epidemic route of this viral disease should be identified and it should be further studied how to stop this virus spreading over the country anymore.

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References

Chung MY, Yoon YI, Hwang JS, Goo TW, Yun EY (2014) Anti-obesity effect of *Allomyrina dichotoma* (Arthropoda: Insecta) larvae ethanol extract on 3T3-L1 adipocyte differentiation. Entomol Res 44, 9-16.

Huger AM (2005) The *Oryctes* virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). J Inverteb Pathol 89, 78-84.

Kim DS, Huh J, You GC, Chae SC, Lee OS, Lee HB *et al*. (2007) *Allomyrina dichotoma* larva extracts protect streptozotocin-induced oxidative cytotoxicity. Environ Health Toxicol 22, 349-355.

Koyama Y, Motobu M, Hikosaka K, Yamada M, Nakamura K, Saido-Sakanaka H *et al*. (2006) Protective effects of antimicrobial peptides derived from the beetle *Allomyrina dichotoma* defensin on endotoxic shock in mice. Int J Immunopharmacol 6, 234-240.

Lee KJ, Lee JB (2009) Protective effect of *Allomyrina dichotoma* larva extract on *tert*-butyl hydroperoxide-induced oxidative hepatotoxicity. Korean J Environ Biol 27, 230-236.

Lee S, Park KH, Nam SH, Kwak KW, Choi JY (2015) First report of *Oryctes rhinoceros nudivirus* (Coleoptera: Scarabaeidae) causing severe disease in *Allomyrina dichotoma* in Korea. J Insect Sci 15, 1-3.

Ramle M, Wahid MB, Norman K, Glare TR, Jackson TA (2005) The incidence and use of *Oryctes* virus for control of rhinoceros beetle in oil palm plantations in Malaysia. J Inverteb Pathol 89, 85-90.

Raubenheimer D, Rothman JM (2013) Nutritional ecology of entomophagy in humans and other primates. Annu Rev Entomol 58, 141-160.

Sagisaka A, Miyanoshita A, Ishibashi J, Yamakawa M (2001) Purification, characterization and gene expression of a glycine and proline-rich antibacterial protein family from larvae of a beetle, *Allomyrina dichotoma.* Insect Mol. Biol 10, 293-302.

Van Huis A (2013) Potential of insects as food and feed in assuring food security. Annu Rev Entomol 58, 563-583.

Wang Y, Bininda-Emonds O, Oers MM, Vlak JM, Jehle JA (2011) The genome of *Oryctes rhinoceros nudivirus* provides novel insight into the evolution of nuclear arthropod-specific large circular double-stranded DNA viruses. Virus Genes 42, 444-456.

Youn K, Kim JY, Yeo H, Yun EY, Hwang JS, Jun M (2012) Fatty acid and volatile oil compositions of *Allomyrina dichotoma* larvae. Prev Nutr Food Sci 17, 310-314.