A survey of *Lasioderma serricorne* (Fabricius) in Japanese Dental Clinics

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This study was to survey the capturing rate in Japanese dental clinics of the *Lasioderma serricorne* (cigarette beetles), and to evaluate the beetle’s potential as a carrier for transmission of nosocomial pathogens. *L. serricorne* imagoes were captured in pheromone traps in 14 Japanese dental clinics in August and September 2012 and 2013, and their numbers recorded. Polymerase chain reaction (PCR) for the bacterial antibiotic-resistant genes *mecA*, *vanA*, *vanB*, *blaIMP*, and *blaVIM* was performed on the captured *L. serricorne* imagoes. Bacterial species in the captured specimens were identified by 16S rRNA PCR and sequencing analysis. The *L. serricorne* imagoes were captured from 10 dental clinics (71.4%). We failed to detect the presence of nosocomial antibiotic-resistant pathogens in *L. serricorne* imagoes. The bacterial species detected most commonly in the imagoes was *Wolbachia* sp., an intracellular proteobacterium infecting certain insect species. Monitoring of insects including *L. serricorne* should be incorporated into regiment of the infection control.

Key words : The cigarette beetle *Lasioderma serricorne* (Fabricius) / Dental clinic / Survey the incidence / Environmental hygiene monitoring.

Insects carrying pathogenic bacteria are found worldwide. In recent years, some insects and mites in Japan have been found to carry dangerous pathogenic bacteria, and deaths in people bitten by ticks and mosquitoes have become a problem (Ministry of Health, Labour and Welfare, 2016; Sajjo, 2014).

*Lasioderma serricorne* (Fabricius) commonly known as the cigarette beetle, is a very small, pale brown beetle (about 2 to 3 mm long). It is distributed worldwide and is a pest of stored tobacco and cigarettes. It often causes serious insect damage, because it is a ubiquitous and destructive pest that feeds on dried foods, including vegetables, cereals, dried fruit, flour, seeds, and some animal products such as dried bonito (Matsuda et al., 2002; Verma, 2011). *L. serricorne* prefers to live in dense urban areas such as schools, hospitals, and shops than in pre-urban and semi-rural areas such as forests (Kawakami and Kase, 1998).

Not only *Aspergillus ochraceus*, which has the ability to produce high toxic ochratoxin, but also *Staphylococcus epidermidis* and *Peptostreptococcus octavius* have been isolated from the abdominal surface of *L. serricorne* imagoes (Kawakami et al., 2002; Kawakami et
The isolation of several bacteria and fungi which can be carried by *L. serricorne* imagos indicates that the beetle is capable of carrying pathogenic bacteria. Accordingly, it may pose serious problems in regard to the transmission and prevalence of infectious diseases.

The aim of this study was to survey the capturing rate of *L. serricorne* imagos in Japanese dental clinics and to evaluate its potential as a carrier for the transmission of nosocomial pathogens.

*L. serricorne* imagos were captured in pheromone traps (New Serrico: Fuji Flavor Co., Ltd.) in fourteen Japanese dental clinics in August and September, 2012 and 2013. Three pheromone traps were set on the wall at height of 1.5 m from the floor around the dental unit in each dental clinic for a month. The every pheromone traps were collected after setting for a month and replaced with new ones. And, the numbers of *L. serricorne* imagos collected every month were recorded.

*L. serricorne* imagos in a pheromone trap are shown in Figure 1, and the numbers of *L. serricorne* imagos captured are shown in Table 1.

The *L. serricorne* imagos were captured from ten of fourteen dental clinics (71.4%). The average number of *L. serricorne* imagos captured in Japanese dental clinics during August and September was 20 (range, 0 to 69) in 2012 and 37 (range, 0 to 150) in 2013.

Further, eight of *L. serricorne* imagos randomly selected from three traps which were set at each dental clinic, collected every month. All eight selected were mixed, and grinded down using a disposable homogenizer with 200 µL InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA). Bacterial DNA was extracted in accordance with the manufacturer’s instructions and used for polymerase chain reaction (PCR).

The antibiotic-resistant bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant *Pseudomonas* (MDRP), extended-spectrum beta-lactamase (ESBL)-producing bacteria, and VRE, were detected by PCR by amplification of the *mecA*, *bla*IMP, *bla*VIM, *bla*TEM, *vanA*, and *vanB* genes, as previously described (Henriques et al., 2006; Kariyama et al., 2000; Murakami et al., 1991).

After being stained with ethidium bromide, the amplified bands were observed under UV transillumination.

The InstaGene Matrix (Bio-Rad, Hercules, CA, USA) was used for DNA extraction from the homogenate of *L. serricorne* imagos.

A 16S rRNA fragment of approximately 600 bp was amplified by PCR using the primers 341f (5’-CCTACGGGA GGC AGC AG-3’) and 907r (5’-CCG TCA ATT CMT TTR AGT TT-3’) (Tsuneishi et al., 2006). The amplified DNA fragments were cloned by using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer’s instructions. DNA sequencing was performed with a BigDye cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an automated DNA sequencer (3130xl Genetic Analyzer; Applied Biosystems). The sequence data were analyzed by using the BLAST sequence homology search program of GenBank. Bacterial species were identified at identity values above 99%.

The results were bacterial antibiotic-resistance genes were not detected in the *L. serricorne* imagos samples. Also, the bacterial species detected most commonly by
In this study, *Wolbachia* sp., *B. cereus* and *B. subtilis* could be detected from the extracts of the captured *L. serricorne* imagoes. However, it was difficult to distinguish their distribution within or on the surface of the insects by these methods. It is necessary to improve the methods for bacterial identification in order to solve this problem.

*L. serricorne* is an urban insect adapted to human life, and it is regarded as a food insect pest because of its predilection for dry foods. In 2012 we collected a total of 285 *L. serricorne* imagoes from Japanese dental clinics, whereas in 2013 we collected 508. Therefore, these clinics are viable habitats of *L. serricorne*.

However, the reason for differences of the captured number of *L. serricorne* imagoes among tested Japanese dental clinics are complex and uncertain, even though it might be dependent on their architectural styles or the cleanliness in dental clinics. In addition, *L. serricorne* may be also due to the airtightness of the building, because it also lives outdoors. In this study, the possible reason that four dental clinic (3~6 in Table 1) had no captured *L. serricorne* imagoes appears 16S rDNA sequence analysis in the imagoes was *Wolbachia* sp., an intracellular proteobacterium known to infect certain insect species. The sequence data were analyzed using the BLAST sequence homology search program at GenBank, and the organism was confirmed to have 100% homology with the published sequence of the *Wolbachia endosymbiont* strain AL01 16S ribosomal RNA gene (Gen-Bank Accession No.KX385013.1) and 100% homology with the *Wolbachia pipiens* strain a.alb6-Marseille 16S ribosomal RNA gene (GenBank Accession KX155506.1). *Wolbachia* sp. was detected in all dental clinics (100%), whereas *Bacillus cereus* and *Bacillus subtilis* were also detected in six clinics (60%) and eight clinics (80%) of the tested ten dental clinics, respectively.

Eight *L. serricorne* imagoes were randomly selected from the captured samples and grinded down all in one piece for the bacterial identification. So, the results of this study could not mention which *L. serricorne* imagoes possessed *Wolbachia* sp., *B. cereus* or *B. subtilis*, but might indicate only bacterial distributions in dental clinics by the *L. serricorne* imagoes.

### Table 1

| No. | Location of Dental Clinic | Architectural styles of Dental Clinic | 2012 | 2013 |
|-----|--------------------------|-------------------------------------|------|------|
| 1   | Okayama Prefecture, Okayama City | A: Steel-framed reinforced concrete building | Aug 17 | Sep 14 | Aug 52 | Sep 14 |
| 2   | Okayama Prefecture, Shoja City | B: Wooden mortar wall building | B: Detached house type clinic | Sep 15 | Aug 1 | Sep 27 | Aug 15 |
| 3   | Okayama Prefecture, Tomata County | C: Reinforced concrete building | C: Building tenant type clinic | C: Dedicated building type clinic | Sep 0 | Aug 0 | Sep 0 | Aug 0 |
| 4   | Okayama Prefecture, Tomata County | B: Wooden mortar wall building | B: Detached house type clinic | B: Dedicated building type clinic | Sep 0 | Aug 0 | Sep 0 | Aug 0 |
| 5   | Okayama Prefecture, Maniwa County | C: Reinforced concrete building | C: Dedicated building type clinic | C: Dedicated building type clinic | Sep 0 | Aug 0 | Sep 0 | Aug 0 |
| 6   | Okayama Prefecture, Tomata County | C: Reinforced concrete building | C: Dedicated building type clinic | C: Dedicated building type clinic | Sep 0 | Aug 0 | Sep 0 | Aug 0 |
| 7   | Okayama Prefecture, Okayama City | B: Wooden mortar wall building | B: Detached house type clinic | B: Dedicated building type clinic | Sep 9 | Aug 1 | Sep 9 | Aug 5 |
| 8   | Okayama Prefecture, Okayama City | B: Wooden mortar wall building | B: Detached house type clinic | B: Dedicated building type clinic | Sep 0 | Aug 2 | Sep 1 | Aug 4 |
| 9   | Kagawa Prefecture, Ayauta Country | A: Steel-framed reinforced concrete building | A: Dedicated building type clinic | A: Dedicated building type clinic | Sep 11 | Aug 10 | Sep 12 | Aug 16 |
| 10  | Hiroshima Prefecture, Mihara City | B: Wooden mortar wall building | B: Detached house type clinic | B: Dedicated building type clinic | Sep 30 | Aug 34 | Sep 49 | Aug 47 |
| 11  | Hyogo Prefecture, Kobe City | C: Reinforced concrete building | C: Dedicated building type clinic | C: Dedicated building type clinic | Sep 16 | Aug 28 | Sep 33 | Aug 31 |
| 12  | Hyogo Prefecture, Amagasaki City | C: Reinforced concrete building | C: Dedicated building type clinic | C: Dedicated building type clinic | Sep 27 | Aug 42 | Sep 60 | Aug 90 |
| 13  | Hyogo Prefecture, Mita City | B: Wooden mortar wall building | B: Dedicated building type clinic | B: Dedicated building type clinic | Sep 11 | Aug 4 | Sep 25 | Aug 8 |
| 14  | Hyogo Prefecture, Kakogawa City | D: Light gauge steel framed building | D: Dedicated building type clinic | D: Dedicated building type clinic | Sep 10 | Aug 3 | Sep 7 | Aug 3 |

The numbers of *L. serricorne* imagoes represent total captured value of the three pheromone traps.
to be due to their location, where are located at highlands in the northern part of Okayama Prefecture. The temperatures are low around there in the summer season when compared to the area in other dental clinics.

Dental treatments often involve surgical procedures using dental instruments (air/water syringes, high-speed turbines, micromotors). They produce sprays containing patient-derived infectious agents such as blood, saliva, and oral microorganisms (Cristina et al., 2008; Perdelli et al., 2008; Rautemaa et al., 2006). Patient blood, saliva and oral microorganisms can be spread as aerosols and droplets during dental treatment; these media are potentially infectious and are sources of cross-contamination (Watanabe et al., 2018). Therefore, it is conceivable that there are many highly pathogenic microorganisms in the dental treatment environment.

In recent years, several fungi and bacteria have been detected on the body surface and in the alimentary canal of *L. serricorne* imagoes, which has therefore attracted attention as a sanitary pest. Therefore, it is assumed the risk that the pathogenic microorganisms of the dental clinics are carried by *L. serricorne* imagoes.

For example, Moth flies *Clogmia albipunctata* (Diptera: Psychodidae) inhabiting hospitals play a role as vectors of pathogenic bacteria (Faulde and Spiesberger, 2013). In addition, antibiotic-resistant bacteria such as MRSA have been isolated from bedbugs and cockroaches (Lowe and Romney, 2011; Soureshjani and Doosti, 2013). In view of nosocomial infection control, the survey of the antibiotic-resistant bacteria is important. In this study, the antibiotic-resistant genes mecA, blalMP, blavIM, blavTEM, vanA, and vanB were not detected in *L. serricorne* imagoes samples. However, it is a concern that *L. serricorne* present in dental treatment environments with high contamination status could still carry a wide range of pathogenic microorganisms. A case of *L. serricorne* spawning and growth in the room has been reported (Miyanoshi et al., 2004). There are dental materials also include dry powders such as those made from seaweed. Therefore, it is likely that *L. serricorne* may feed them, and propagate itself in dental clinics. We therefore consider it necessary to pay full attention to the life cycle of *L. serricorne*. Growth is related to temperature: in Japan, the population of *L. serricorne* is highest in summer, when it is warmest (Nakan, 2000). In addition, *L. serricorne* is tolerant of otherwise-lethal high temperatures of 50°C; high temperatures are sublethal to all stages of its life cycle (egg, pupa, larva, adult) (Li et al., 2018). In areas or countries with high ambient temperatures it is therefore possible that *L. serricorne* will survive and grow. Furthermore, changes in temperature and precipitation due to abnormal weather conditions can affect the abundance and distribution of bacterial carriers such as *L. serricorne* and thus have serious implications for the transmission and prevalence of infectious diseases.

In this study, it was revealed that *L. serricorne* imagoes invaded many Japanese dental clinics. There are many places in the medical environment where there is insufficient hygiene maintenance by visual confirmation. Therefore, we would like to propose that surveys of monitoring of insects including *L. serricorne* should be incorporated into infection control regimens.

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