Comparison of Prostaglandin E2 (PGE2) in Salivary Gland of *Boophilus microplus*, *Haemaphysalis longicornis* and *Ixodes holocyclus*, and Quantification of PGE2 in Saliva, Hemolymph, Ovary and Gut of *B. microplus*

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**ABSTRACT.** The amount of prostaglandin E2 (PGE2) in salivary gland of semi-engorged adult female *Boophilus microplus*, *Haemaphysalis longicornis* and *Ixodes holocyclus* were 374.3 pg, 427.0 pg and 825.0 pg per one tick, respectively. It was thought that the PGE2 production is a common phenomenon among ticks. Then PGE2 concentrations in saliva and hemolymph, salivary gland, ovary and gut of fully-engorged adult female *B. microplus* were compared. The PGE2 concentration in saliva induced by piocarpine was 40.3 ng/ml and hemolymph was 19.1 ng/ml. Salivary gland, ovary and gut from a tick contains 35.5 pg, 27.0 pg and 2.5 ng of PGE2, respectively.—**KEY WORDS:** prostaglandin E2, salivary gland, tick.

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Tick saliva contains many kinds of substances such as prostaglandins or enzymes which may be beneficial to infestation of ticks on their host [1–6, 9–12]. Prostaglandin E2 (PGE2) is believed to be the major component that contributes to the immunosuppression induced by *Boophilus microplus* [4, 5] and *Ixodes dammini* (syn. *I. scapularis*) [9, 10]. Inokuma *et al.* [5] found that a semi-engorged female *B. microplus* could transmit 1.8 ng PGE2 into the feeding site and it seemed sufficient to suppress the response of bovine mononuclear cells to mitogens. This effect of PGE2 is thought to play an important role in host response to the transmission of tick-borne diseases [4, 5, 9–11], but there have been few information available about the PGE2 production from ticks. We wish to report some new evidences that are concerned with the PGE2 production.

In Australia, *B. microplus*, *H. longicornis* and *I. holocyclus* are all veterinary importance as vectors of tick-borne diseases. *H. longicornis* is also a common tick in Japan and it transmit pathogens of Theileriosis [8]. However, the salivary prostaglandins in *H. longicornis* and *I. holocyclus* have not been studied. Accordingly, we measured the level of PGE2 in salivary gland of semi-engorged adult female *H. longicornis* and *I. holocyclus* as well as *B. microplus* to decide wether the PGE2 production is a common phenomenon among ticks.

Prostaglandin activity was also found in hemolymph of *B. microplus* [2] and reproductive organs of *Hyalomma anatolicum excavatum* [12], but there are few quantitative studies about PGE2 in other organs. We measured PGE2 in saliva, hemolymph, salivary gland, ovary and gut of fully-engorged adult female of *B. microplus* by radioimmune assay to study the distribution of PGE2 in ticks.

The Yeerongpilly laboratory strain of *B. microplus* and *H. longicornis* were fed on cattle and were picked off when they had reached 4–6 mm in length (semi-engorged adult female). Fully-engorged *B. microplus* were removed from cattle. Semi-engorged adult female *I. holocyclus*, which were picked off from laboratory rats, were kindly given from Dr. B. F. Stone (a former scientist of CSIRO, Long Pocket Laboratories). Salivary glands, ovaries and guts from ticks were dissected and washed once in cold phosphate buffered saline (PBS, pH 7.2) followed by homogenizing in PBS at 0°C using a glass homogenizer. Then these samples were ultrasonicated with small probe (3 mm) for 2 min using a rapid ultrasonic disintegrator (Ultrasonics Ltd., U.S.A.). Homogenized organs were centrifuged at 2,000 rpm for 5 min to remove large debris. Saliva was collected from fully-engorged adult females of *B. microplus* using the method of Kerlin and Hughes [7]. Hemolymph was collected into micro-capillary tubes coated with EDTA (0.15 M) from fully-engorged adult females of *B. microplus* by cutting legs with fine scissors. Samples were purified and methyl oximated, followed by PGE2 were measured using a prostaglandin E2 [125I] assay system (Batch No. L41A, Amersham, U.K.), as described by the manufacturer.

The amount of PGE2 in salivary gland of a semi-engorged adult female of *B. microplus*, *H. longicornis* and *I. holocyclus* were shown in Table 1. The amount of PGE2 of *I. holocyclus* were significantly larger than *B. microplus* (p<0.02) and *H. longicornis* (p<0.001). The difference of PGE2 level between *B. microplus* and *H. longicornis* was not significant. At this stage, *B. microplus* could transmit sufficient amount of PGE2 into the feeding site to suppress the bovine mononuclear cells to mitogens [5]. It is suspected by these finding that *H. longicornis* and *I. holocyclus* may transmit sufficient amount of PGE2 into the feeding sites to produce physiological effects on the bovine host as well as *B. microplus*. Prostaglandins are also found in saliva or salivary gland of *Amblyomma americanum* [11] and *H. anatolicum excavatum* [12]. It seems that the PGE2 production is a common phenomenon among ticks.

The PGE2 concentration of saliva and hemolymph from fully-engorged adult female *B. microplus* were 40.3±6.8

<table>
<thead>
<tr>
<th>Tick</th>
<th>Prostaglandin E2 (pg/tick)</th>
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<tbody>
<tr>
<td><em>Boophilus microplus</em></td>
<td>374.3± 46.9 (9)</td>
</tr>
<tr>
<td><em>Haemaphysalis longicornis</em></td>
<td>427.0± 101.0</td>
</tr>
<tr>
<td><em>Ixodes holocyclus</em></td>
<td>825.0± 15.0</td>
</tr>
</tbody>
</table>

a) Mean±standard error of results from 3 experiments. Each experiment used 10 to 30 ticks.
Table 2. Prostaglandin E2 in saliva, hemolymph, salivary gland, ovary and gut from fully-engorged adult female Boophilus microplus

<table>
<thead>
<tr>
<th>Organs</th>
<th>Prostaglandin E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva</td>
<td>40.3± 6.8 ng/ml</td>
</tr>
<tr>
<td>Hemolymph</td>
<td>19.1± 6.1 ng/ml</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>35.5±10.5 pg/tick</td>
</tr>
<tr>
<td>Ovary</td>
<td>27.0 pg/tick</td>
</tr>
<tr>
<td>Gut</td>
<td>2.5 ng/tick</td>
</tr>
</tbody>
</table>

ng/ml and 19.1±6.1 ng/ml as shown in Table 2. Salivary gland, ovary and gut from a fully-engorged adult female B. microplus contained 35.5±10.5 pg., 27.0 pg and 2.5 ng, respectively (Table 2). Dickinson et al. [2] found that salivary gland and hemolymph of semi-engorged females of B. microplus had prostaglandin equivalent activities. They measured the activity by using the smooth muscle contracting method, while we measured PGE2 by radioimmune assay, which was more specific and sensitive method. Shemesh et al. [12] also found that salivary gland and reproductive organs of H. anatolicum excavatum produced PGE2 in vitro. In our study, it was shown that hemolymph, ovary and gut from fully-engorged adult female B. microplus also contained PGE2 as well as saliva or salivary gland. The existence of PGE2 in hemolymph may suggest that the possibility of circulation of PGE2 inside the tick body. The amount of PGE2 in gut sample was comparatively high. Gut which was dissected from ticks contained digested host blood with tick’s own cells. A. americanum may obtain arachidonic acid from the bloodmeal and synthesize prostaglandins by itself [11]. It was also possible that PGE2 of gut came from host blood and concentrated inside B. microplus.

Our present experiments demonstrates that semi-engorged adult female H. longicornis and I. holocyclus contain PGE2 in their salivary gland as well as B. microplus and that PGE2 is also existed in hemolymph, ovary and gut from engorged adult female of B. microplus.

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REFERENCES