Efficacy of Naphthoquinones and Imidocarb dipropionate on *Theileria sergenti* Infections in Splenectomized Calves

Tetsuro MINAMI, Terushi NAKANO\(^1\), Shinya SHIMIZU, Kameo SHIMURA, Toru FUJINAGA, and Shingo ITO

*First Research Division, National Institute of Animal Health, Kannondai, Yatabe, Tsukuba-gun, Ibaraki 305, and 1*Koibuchi Agricultural College, Koibuchi, Uchiara, Higashi-ibaraki, Ibaraki 319-03, Japan

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**ABSTRACT.** Twelve splenectomized calves infected with *Theileria sergenti* were treated with naphthoquinones (parvaquone or 720C) or imidocarb. Both parvaquone and 720C gave a good anti-intraerythrocytic piroplasm activity with no side effects. 720C appeared to be more effective than parvaquone. In the imidocarb treated calves, however, there were irregular responses and treatment with more than 6 mg/kg resulted in some side effects. In 4 calves treated with a single dose of 2.5–10 mg/kg of 720C, parasitemia fell to nil during the 9 days after treatment and no piroplasms were detected for the next 16–40 days.—**KEY WORDS:** *Theileria sergenti*, naphthoquinones.

Theileriosis caused by *Theileria sergenti* is a major threat to grazing cattle in Japan [2]. Holstein cattle, introduced to a pasture for the first time, usually show a higher parasitemia than Japanese Black cattle, although both breeds show clinical disease [3]. The disease control has been based on the use of acaricides and protozoacides. The 8-aminoquinoline compounds (pamaquine and primaquine) are still in wide use as anti-intraerythrocytic piroplasm drugs. However, it is said that their anti-piroplasm activity has been declining gradually under field conditions. This paper describes the anti-piroplasm activity of the naphthoquinones (parvaquone and 720C) and imidocarb against *T. sergenti* infections.

Twelve Holstein calves between 3 and 6 months of age were used and were splenectomized before use. Calf 6 was infected with the Ikeda strain of *T. sergenti* isolated in 1982 from *Haemaphysalis longicornis* ticks collected from grazing cattle in a infected pasture in Tochigi prefecture. Calves 8 and 9 were infected naturally with *T. sergenti*. The other 9 calves were infected with the Fukushima strain [5]. Ground-up tick supernates were used to infect calves 3, 5, 6 and 12. The other 6 calves except calves 8 and 9 were given infected blood.

Two naphthoquinones, parvaquone (Clexon) and 720C, were obtained from the Wellcome Foundation Ltd. For comparison, imidocarb dipropionate (Imizol, Wellcome Foundation Ltd.) and pamaquine (Oil pamaquine, Nippon Zenyaku Kogyo Co. Ltd.) were used. Treatment was given when the piroplasm parasitemia reached 2.3–29.0%. Two calves were treated with a dose of 10 and 20 mg/kg (12.7–25.3 ml) of parvaquone intramuscularly, 5 calves with 1.25–10 mg/kg (3.0–15.4 ml) of 720C intramuscularly, 4 calves with 2.4–15 mg/kg (2.0–13.8 ml) of imidocarb subcutaneously and one calf with 4 mg/kg (2.0 ml) of pamaquine intramuscularly (Table 1). Clinical, hematological and serological examinations were carried out daily, or at 1–7 days intervals. Red blood cell (RBC) counts and mean corpuscular volume (MCV) were determined by using a Coulter counter (Model ZF and Model MHR,
Table 1. Anti-piroplasm activity of parvaquone, 720C and imidocarb

<table>
<thead>
<tr>
<th>Drug</th>
<th>Calf number</th>
<th>Dose (mg/kg)</th>
<th>Parasitemia at treatment (%)</th>
<th>Days after treatment to nil parasitemia</th>
<th>Persistence of nil parasitemia, days</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parvaquone</td>
<td>1</td>
<td>10.0</td>
<td>12.4</td>
<td>Still present</td>
<td>No effect</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20.0</td>
<td>11.3</td>
<td>2</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.25</td>
<td>4.5</td>
<td>Still present</td>
<td>Slightly effective b)</td>
<td>–</td>
</tr>
<tr>
<td>720C</td>
<td>4</td>
<td>2.5</td>
<td>5.5</td>
<td>9</td>
<td>21</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.0</td>
<td>5.3</td>
<td>2</td>
<td>27</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5.0</td>
<td>23.2</td>
<td>3</td>
<td>16</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>10.0</td>
<td>7.0</td>
<td>3</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td>Imidocarb</td>
<td>8</td>
<td>2.4</td>
<td>2.3</td>
<td>Still present</td>
<td>Slightly effective c)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>6.0</td>
<td>29.0</td>
<td>12</td>
<td>15</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.0</td>
<td>9.2</td>
<td>Still present</td>
<td>Slightly effective d)</td>
<td>+</td>
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<tr>
<td></td>
<td>11</td>
<td>15.0</td>
<td>9.8</td>
<td>6</td>
<td>27</td>
<td>+</td>
</tr>
<tr>
<td>Pamaquine</td>
<td>12</td>
<td>4.0</td>
<td>4.1</td>
<td>3</td>
<td>20</td>
<td>–</td>
</tr>
</tbody>
</table>

a) No. 6: Infected with Ikeda strain, No. 8 and 9: naturally, the other calves: with Fukushima strain.
b) Parasitemia fell to below 0.1% on day 3 after treatment and the same level lasted for 18 days.
c) Parasitemia fell to below 0.1% on day 10 after treatment and the same level lasted for 3 days.
d) Parasitemia fell to below 0.1% on day 10 after treatment and the same level lasted for 7 days.

Fig. 1. Hematological and serological changes following treatment with 720C in a splenectomized calf experimentally infected with *T. sergenti*. 
Coulter Electrics, Inc.). Significant negative parasitemia was determined by the examination of at least 100 microscopic fields, or $5 \times 10^4$ of RBC. The indirect fluorescent antibody (IFA) test [8] and the complement fixation (CF) test [5] were performed for checking serological changes in the course of *T. sergenti* infections.

The degree of an efficacy of four different drugs was shown in Table 1.

Calf 1 was treated firstly with a dose of 10 mg/kg of parvaquone, but the piroplasms did not disappear. The same animal was given additional treatment, with a dose of 20 mg/kg, on day 6 after the first treatment. As a result, a rapid disappearance of piroplasms was observed and no parasites were detected from the peripheral blood for 7 days. The same result was obtained from the trial on calf 2.

Calf 3, treated with a dose of 1.25 mg/kg of 720C, did not show disappearance of piroplasms. However, parasitemia fell to below 0.1% on day 3 after treatment and did not exceed 0.1% for 18 days.

In calf 4 treated with a dose of 2.5 mg/kg of 720C, it took 9 days for parasitemia to nil. However, most of piroplasms on day 1 to 8 after treatment degenerated morphologically. After that no parasites were found for the following 21 days in this calf.

Parasitemia in calves 5, 6 and 7, treated with 720C, 5 and 10 mg/kg, fell rapidly and disappeared by the third day after treatment and no piroplasms were found for the next 16–40 days.

Calf 6 was treated with a dose of 5 mg/kg of 720C when clinical and hematological changes were severe. As shown in Figure 1, parasitemia fell from 23.2% to nil by the third day after treatment, and clinic-hematological changes also recovered rapidly. Serologically, marked differences were not detected between pre-treatment and post-treatment during the 30 days tested.

The imidocarb treatment resulted in irregular responses, and its effect was limited. In the animals treated with more than 6 mg/kg of imidocarb, marked side effects, i.e. excessive salivation, rapid breathing and uneasiness, were observed for at least 30 to 120 minutes.

Calf 12, treated with a dose of 4 mg/kg of pamaquine, showed a rapid reduction of parasitemia and no piroplasms were found for 20 days.

Parvaquone is an effective drug against schizonts of *T. parva* and *T. annulata* [1, 4]. However, there are very few experiments on its anti-piroplasm activity. The relapse interval of piroplasms is very short, although a rapid reduction of parasitemia is shown in a parvaquone treated calf infected with *T. mutans* [1]. The same pattern was observed in parvaquone treated calves infected with *T. sergenti*.

Efficacy of imidocarb against *T. sergenti* piroplasm has also been reported [6, 7]. From our experiments using splenectomized calves infected with *T. sergenti*, it was considered that the therapeutic dose for babesiosis was insufficient against *T. sergenti* infecton. However, a slow reduction of parasitemia was observed. Imidocarb, therefore, might be effective in a pasture contaminated with the piroplasmosis syndrome, as pointed out by Purnell and Rae [7].

In this experiment, it was found that 720C, a naphthoquinone derivative, showed a strong anti-piroplasm activity in *T. sergenti* infection. Also no side effects were determined clinically. Consequently more detailed examinations, e.g. effective dose range, efficacy in the field, prophylactic activity, appearance of drug resistance and compatibility with other drugs, are required before its practical use.

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T. Kamio of the National Institute of Animal Health for providing the Ikeda strain of *T. sergenti*, and the Kyoritsu Shoji Co., Ltd. for providing the naphthoquinones and the imidocarb dipropionate for these experiments.

REFERENCES


要約

ナフトキノーン製剤及びイミドカルプの抗タイリリア効果（短報）: 南 哲郎, 中野光志, 清水真也, 志村亀夫, 藤永徹, 伊藤進午（農林水産省家畜衛生試験場, 1鹤岡学園）—— *Theileria sergenti* 感染卵殖子牛を用いて, ナフトキノーン製剤（パルバックン, 720C）及びイミドカルプの抗タイリリア効果を調べた。パルバックン及び720Cは赤血球型原虫に対して即効性を示したが, イミドカルプは強効性で, 6 mg/kg 以上の応用では副作用を示した。720Cについて寄生率が5.3～23.2%に達した時に2.5～10mg/kg を筋肉内に1回注射したところ, 赤血球型原虫は9日以内に顕著に, 那消失期間は16～40日であった。