Invasion of Bovine Erythrocytes by *Theileria sergenti* Piromasms in vitro

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Intraerythrocytic stage (piroplasm) of *T. sergenti* multiplies in the bovine erythrocytes, when pyrexia and anemia are aggravated [4]. But it is not clear whether the intraerythrocytic stage multiplies only from the exoerythrocytic stage, or piroplasms escape from host cells and penetrate into intact erythrocytes. Higuchi et al. [2] suggested the correlation between the forms of the intraerythrocytic stage and the multiplication of *T. sergenti* in cattle. The multiplication mode of piroplasm stage should be immediately cleared for the control of *T. sergenti* infection. In this study we tried to elucidate the invasion of bovine erythrocytes by piroplasms of *T. sergenti*.

Intraerythrocytic stage of *T. sergenti* was isolated by the modified methods reported in a previous paper [6]. Viable parasites isolated from erythrocytes were counted by staining with acridine orange [3]. Parasites were adjusted in number to 1×10³/ml of minimum essential medium (MEM) added with 10% heat-inactivated fetal calf serum (FCS). Washed intact erythrocytes were suspended in MEM with 10% FCS to give 5% cells to the medium. Ten parts of free parasite suspensions were mixed with 1 part of erythrocyte suspension, and incubated at 37°C for 10, 30, 60 min, 3, 6, 12, 24, 48 and 72 hours. A few drops of the mixture were sampled from the preparations at each incubation time, and examined by light microscopy after stained with Giemsa (pH6. 8, PBS) and by transmission electron microscopy (TEM).

A splenectomized Holstein calf, 4 months old, was intravenously inoculated with isolated piroplasms (3×10³) to determine their infectivity. Piroplasms were detected from the peripheral erythrocytes of the inoculated calf on the 31st day after inoculation. Subsequently, the typical clinical signs of Japanese thileriosis developed. Therefore, the intraerythrocytic stage of *T. sergenti* could infect the calf.

The isolated piroplasms of *T. sergenti* completely invaded erythrocytes in vitro within 10 min. Most of the parasites that invaded blood cells were the ring form with the ubiquitous crescent basophilic portion and the lucent cytoplasm (Fig. 1). The observation by TEM revealed that piroplasms completely invaded erythrocytes. A great deal of free ribosomes and the cytostome were found in the parasite cytoplasm (Fig. 2). The rate of erythrocytes invaded by parasites was extremely low ranging 0.2~0.8% (n=7), and did not markedly change with incubation time. Serial invasive process of erythrocytes by isolated parasites was observed at each incubation time (Figs. 3 and 4). Piroplasms consistently penetrated into the erythrocyte by the anterior basophilic portion during the invasive process. The basophilic portion was the nuclear hemisphere of parasite (Fig. 5).

The majority of parasites attached themselves only to the erythrocytes, and varied in shape, such as rod and comma shaped, spherical, and ovoid (Fig. 6). The rate of erythrocytes attached by parasites did not change at the shorter incubation time (15~60 min), but tended to

![Fig. 1. Invaded erythrocyte by a piroplasm 10 min after incubation (arrow head). Scale bar=10 μm.](image-url)
increase at the longer incubation time (3~72 hrs). Most of attaching parasites did not invade the erythrocytes, and ultimately detached themselves from the erythrocytes.

*Haemoproteid* parasites penetrate into host cells without the rupture of host cell membrane [1, 5, 8]. The invasion mechanisms of host cells by parasites were profoundly investigated [1, 5, 8]. *Babesia* [8] and *Plasmodium* [1] attach themselves to the membrane of host cell by the anterior end and start to invade the cell. But the sporozoites of *T. annulata* attached themselves to the host cell membrane by the basal end (nuclear
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hemisphere) which acts as the specific receptor site [5]. In the case of *T. sergenti*, piroplasms entered the erythrocytes by the nuclear hemisphere. Both piroplasms of *T. sergenti* and sporozoites of *T. annulata* are similar in the specific receptor site. This suggests that the interiorization between parasites and host cells is subsequently facilitated.

It is suggested that piroplasms of *T. mutans* could multiply in the peripheral blood circulation in the absence of schizonts [7]. The isolated piroplasms of *T. sergenti* had the ability to infect cattle in vivo, and invaded the erythrocytes in vitro. *T. sergenti* might be able to multiply in cattle in the absence of schizonts after micromerozoites penetrate into the erythrocytes.

At present, the studies on the infection with *T. sergenti* are usually made by the experimental inoculation of cattle, which is too time consuming, expensive, and troublesome to be carried out. *T. sergenti* piroplasms isolated in vitro from the infected erythrocytes will be used in the experiments to clarify the multiplication mechanisms of the parasite.

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REFERENCES


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*Theileria sergenti* 赤内型原虫による赤血球への侵入（短報）：川本 哲・高橋清志・小沼 裕1)・黒沢 隆・其田三夫（駒薬学園大学獣医内科学教室、1)北海道大学家畜伝染病学教室）——*T. sergenti* 感染赤血球からピロプラズムを単離し、in vitro において赤血球内への侵入実験を行った結果、少数の原虫が10分以内に赤血球内へ侵入を完了したが、多数の原虫は赤血球の表面に付着したままで侵入しなかった。その赤血球侵入率は0.2〜0.8%（n＝7）であった。