Theileria sergenti: A Simple Method for Isolation of Piroplasms from Erythrocytes

Kozo FUJISAKI, Tsugihiko KAMIO, Yoshio NAKAMURA, Kameo SHIMURA, Yukio TAKAHASHI, Shin'ichiro KAWAZU, Shinya SHIMIZU, Tetsuro MINAMI, and Shingo ITO
First Research Division, National Institute of Animal Health, 3–1–1, Kannondai, Tsukuba, Ibaraki 305, and
Hokubu Livestock Hygiene Service Centr., 92, Wakigami, Takanosu, Kita-akita-gun, Akita 018-34, Japan
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Immunological, biochemical and other studies of intraerythrocytic parasites would be greatly facilitated if the parasites could be obtained free from erythrocytes. Several methods have been applied for the isolation of the erythrocytic stages, piroplasms, of Theileria sergenti which is the causative agent of bovine theileriosis in Japan. At present the nitrogen cavitation method by Shimizu et al. [5] is regarded as the best way for the isolation piroplasm without contaminants. However, this method requires the expensive and specialized apparatus, and is somewhat difficult to apply for small amount of blood less than 10 ml. We devised a simple and quick method for isolating piroplasms of T. sergenti from less than 10 ml of infected blood. The technique uses the clot of naturally coagulated blood from cattle with piroplasms; and no special equipments.

One splenectomized calf was infected with Ikeda stock of T. sergenti [1] by the infestation of 200 infected nymphal Haemaphysalis longicornis ticks.

From 5 to 10 ml of blood was collected from the calf showing parasitaemia over 5% (the percentage of parasitized erythrocytes in 1,000 erythrocytes) and settled down for 1 to 2 hr at room temperature for producing a clot. In order to release and harvest piroplasms the cell filtration apparatus for tissue culture (RKI Cell Filtration Apparatus Type 135, Ikemoto-Rika Co., Tokyo) was used. The clot was placed on a sheet of 50-mesh wire gauge of this apparatus and cut into pieces of 2 to 3 mm with sharp scissors (Fig. 1). These pieces were then pressed against mesh with a rubber rod pouring PBS (200 mM sodium phosphate, pH 7.2, 150 mM NaCl) or RPMI medium of 20 to 30 ml. The suspension of filtrated erythrocytes was transferred into a 20 ml syringe. A membrane filter holder containing a Millipore filter (pore size 1.2 μm) was attached to a syringe and the suspension of erythrocytes was expelled through this sieve using only slight pressure. The resultant filtrate enriched with freed parasites was centrifuged for 20 min at 47 g at 4°C to remove contaminants such as intact erythrocytes and cell debris. The supernatant was centrifuged again for 30 min at 3,000 to 3,400 g at 4°C to harvest freed piroplasms and the pellet was resuspended in 100 μl of PBS or RPMI medium. The Giemsa-stained smears of the pellet were shown in Fig. 2 and piroplasms appeared free of contamination by erythrocytes while small amount of cell debris were observed.

Two hundred μl of isolated piroplasm suspension was homogenized in a glass homogenizer in an ice bath with the equal volume of cold distilled water 1 mM EDTA. The resulting homogenate was electrophoresed using disc polyacrylamide gel and examined for lactic acid dehydrogenase (LDH) isoenzyme [8]. The result was that one band which appeared to be parasite associated was observed in addition to four bands originated from host cells. Furthermore, the isolated piroplasm suspension was successfully used as antigens in an indirect immunofluorescent test [7], and an enzyme linked immunosorbent assay (ELISA) [5]. Fig. 3 shows the immunofluorescence of freed piroplasms. In ELISA, the optimal dilutions of the antigen preparation and the conjugate were 1:4,000 when the protein concen-

![Fig. 1. The cell filtration apparatus for harvesting tissue cells.](image-url)
Fig. 2. The Giemsa-stained smear of the suspension of freed *Theileria sergenti* piroplasms. ×2,100. (Freed piroplasms appeared to be poor in cytoplasm and karyopycnotic.)

Fig. 3. The result of indirect immunofluorescent test used freed *T. sergenti* piroplasms as antigens. ×1,600.

...tration in piroplasm suspension was 4.93 mg/ml.

The passage through the sieves of erythrocytes agglutinated with Concanavalin A have been used for harvesting merozoites of several species of *Plasmodium* [2, 3, 4]. There are, however, no reports using a clot for sieving. Since erythrocytes of uncoagulated blood can be passed through a Millipore filter (pore size 1.2 μm) as the intact state, the erythrocyte membrane after coagulation might be ruptured easily by the physical stimuli [6] and release the cell contents such as hemoglobin and parasites. It was consid-
erated that piroplasms isolated from a clot might preserve well the isoenzyme activity and the antigenicity for serological tests, and could be applied for the various studies on T. sergenti. However, it was apparent from the microscopy and electrophoresis that the contaminants from host cells have been still included in the final harvest of piroplasms. It is, thus, necessary to contrive the technique for the separation of freed parasites.

REFERENCES


要約

血餅を用いた Theileria sergenti のピロプラズマの簡易分離法（短報）：藤崎幸蔵・池尾次彦・中村義男・志村亀夫・高橋幸男 1)・河津信一郎・清水真也・南哲郎・伊藤進午（農水省家畜衛生試験場，秋田県北部家畜保健衛生所）——小型ピロプラズマ原虫 Theileria sergenti の感染血液の血餅を、細切後にミリポアフィルター（孔径 1.2 μm）通過することによって、ピロプラズマを容易に分離できた。得られた分離ピロプラズマは酵素抗体法用の抗原として使用でき、また原虫のアイソエンザイム活性を保持していることが示された。