High Susceptibility of Djungarian Hamsters (*Phodopus sungorus*) to the Infection with *Babesia microti* Supported by Hemodynamics

Kazunori IKE1), Tetsuya KOMATSU1), Takashi MURAKAMI1), Yousuke KATO1), Miwa TAKAHASHI1), Yuko UCHIDA1) and Soichi IMAI1)

1)Department of Veterinary Parasitology, Nippon Veterinary and Animal Science University, Musashino, Tokyo 180–8602, Japan

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**ABSTRACT.** As the comparative study was carried out on the susceptibility by the pursuit of parasitemia among the Djungarian, Syrian, and Chinese hamsters as well as BALB/c mice infected with the Syrian hamster-adapted *Babesia microti* strain, and Djungarian hamsters showed the highest parasitemia among them. Then, the other hematological parameters were pursued in the Djungarian hamsters infected with the hamster-adapted *B. microti* strain. Remarkable symptoms observed were hemoglobinuria clinically, anemia hematologically, and splenomegaly macroscopically during all over the observation period for 24 weeks post infection (PI). Parasitemia began to rise at 2 weeks and peaked at 4 weeks PI. After that, parasitemia decreased gradually but was maintained with a level of about 10% on average until 24 weeks PI at the end of the experiment. A decrease in the RBC count, Hb, and PCV, and an increase in the reticulocyte and WBC counts due to the development of immature neutrophils, lymphocytes and monocytes were recognized together with a rise of parasitemia. The hamsters had macrocytic hypochromic anemia due to the increase of MCV and the decrease of MCHC in the growth phase of the parasite. It was considered that the Djungarian hamsters will be useful for the infection examination, isolation, maintenance, and passage of *B. microti* in laboratory.

**KEY WORDS:** *Babesia microti*, Djungarian hamster, hematology, *Phodopus sungorus*, susceptibility.

Some protozoa of the genus *Babesia* represent some parasites infective to wild and domestic animals [12]. They require susceptible invertebrate and vertebrate hosts, such as several tick species and mammals, to maintain their life cycles in the field [9]. Several species of them are known to infect humans, in which *B. microti* is the most frequently identified species [6]. The distribution area of human cases of babesiosis caused by *B. microti* is constantly defined, although the human cases of the parasitosis was originally identified in the several areas of northeastern and midwestern United States [19]. Human babesiosis by the parasite has been reported in New Jersey in the United States [3, 21], Europe [3, 10], and Japan [16, 17]. Small rodents play a role of reservoir host of the parasite [18], which are generally show parasitemia infection and only chronic infection with less than 0.5% infected erythrocytes in the enzootic regions [1, 11]. For the experimental infection with common pathogenic parasites, BALB/c mice are the most commonly used, but they have only low susceptibility to *B. microti* [19]. Syrian hamsters (*Mesocricetus auratus*) have high susceptibility to *B. microti* infection and are used to isolate and maintain the parasites in laboratories [4, 18], but complicated works and large space are necessary to keep many hamsters. On the other hand, the Djungarian hamster (*Phodopus sungorus*) of dwarf size is one of the useful rodent host for infection model with *Neospora caninum* [20], although there is no report on *B. microti* infection. Djungarian hamsters have such characteristics as high incidence of neoplasia, susceptibility to carcinogens, and infectivity to oncogenic viruses such as Rous sarcoma virus, human adenovirus-12, and simian virus 40 [9]. In addition, the hamsters are easily bred and kept with comfortable handling.

In this examination, we compared the susceptibility of the Djungarian hamsters to *B. microti* infection with those of Syrian and Chinese hamsters (*Cricetulus griseus*) and BALB/c mice (*Mus musculus*) based on the level of parasitemia and also examined on the other hematological parameters.

**MATERIALS AND METHODS**

**Laboratory animals:** Female Syrian hamsters, 5-week-old, and female BALB/c mice, 5-week-old, were purchased from Saitama Experimental Animal Supply Co., Ltd. (Saitama, Japan). Djungarian and Chinese hamsters used were 10-week-old females and maintained in the laboratory animal facility at Nippon Veterinary and Animal Science University.

**Parasite:** *B. microti* strain AJ used was isolated from a human patient at Harvard University in the United States and maintained by blood passages with Syrian or Djungarian hamsters in Nippon Veterinay and Animal Science University.

**Infection of the experimental animals with the parasite:** *B. microti* isolate was interperitoneally inoculated to the animals at a dose of $1 \times 10^7$ parasitized erythrocytes per head, which were collected from Syrian and Djungarian hamsters infected with the parasite for experiment (Exp) 1 and 2, respectively.

**Parasitemia:** Parasitemia was monitored in the peripheral circulation throughout the observation period by preparing a
thin blood smear each with a drop of blood obtained from hamsters and mice at the tip of infraorbital and tail veins, respectively. Then the percentage of parasitized erythrocytes was determined by counting at least 1,000 erythrocytes per smear stained by Diff-Quik (International Reagents Corporation, Hyogo, Japan).

Exp 1: Comparison of parasitemia in the hamsters and mice: Ten animals each of Djungarian, Syrian and Chinese hamsters, and ten BALB/c mice were infected with *B. microti* and examined for their parasitemia once a week until 10 weeks post infection (PI).

Exp 2: Hemodynamics of the Djungarian hamsters infected with *B. microti*: The hemodynamics during the course of infection in the Djungarian hamsters infected with *B. microti* were examined. Ten *B. microti*-infected and non-infected (control) Djungarian hamsters each were examined once a week until 24 weeks PI for hematological parameters. The infected Djungarian hamsters was examined for parasitemia according to the method above-mentioned. Blood was collected from the hamsters at the infraorbital vein. Erythrocyte (RBC) and leukocyte (WBC) counts, hemoglobin (Hb), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were determined in the hamsters using the mouse mode of Celltacα (Nihon Kohden, Tokyo, Japan). Total protein (TP) was determined with protein refractometer (ATAGO, Tokyo, Japan). Reticulocytes, neutrophils, eosinocytes, basophilocytes, lymphocytes and monocytes were calculated under the microscope.

Statistical analysis: Differences between the two groups, *B. microti*-infected and non-infected groups, were determined by means of Student’s *t*-test. Values of *P*<0.05 were regarded as significant.

RESULTS

*Parasitemia in Exp 1*: Fluctuations of average (Av) parasitemia in the three species of hamsters and BALB/c mice infected with *B. microti* are shown in Fig. 1. Djungarian hamsters had the higher susceptibility than other hamsters and BALB/c mice. In both Djungarian and Syrian hamsters, a rise in parasitemia was recognized at 1 week PI, and the highest Av parasitemia was 56.9% and 49.5%, respectively, at 4 weeks PI. These hamsters successively showed high parasitemia (more than 20%) until the end of the examination (10 weeks PI) and parasites were detected from the peripheral blood. On the other hand, in Chinese hamsters, parasitemia first rose at 3 weeks PI, peaked at 4 weeks PI (23.6% on Av), and then declined rapidly, at the Av values of parasitemia were 3.2%, 2.4%, 1.6%, 1.5%, and 2.1% at 6, 7, 8, 9 and 10 weeks PI, respectively. In BALB/c mice, only a small number of parasitized erythrocytes were detected in the peripheral blood.

*Clinical symptom in Exp 2*: In the Djungarian hamsters, a remarkable clinical symptom, hemoglobinuria, was observed from 4 to 24 weeks PI. Macroscopically, the hamsters had splenomegaly at necropsy performed at 24 weeks PI (Fig. 2).

*Hematological findings in Exp 2*: The pattern of parasitemia observed in the Djungarian hamsters is shown in Fig. 3. *B. microti* merozoites first appeared at 2 weeks PI in the peripheral blood of the hamsters (8.6% on Av). The Av value of parasitemia progressively increased and peaked synchronously in all the hamsters at 4 weeks PI (54.4% on Av). The Av parasitemia subsequently declined to 38.0% at 6 weeks PI, and to 19.0% at 8 weeks PI. At the end of experiment in 24 weeks PI, the parasites were still detected in the peripheral blood of the hamsters (9.2% on Av).

The minimal RBC counts (5.2 × 10⁶/µl on Av), PCV (31.5% on Av) and Hb values (8.5 g/dl on Av) were recognized in the hamsters, when the level of parasitemia became the highest (Figs. 4A, 4B, and 4C). Though these parame...
Susceptibility in Djungarian Hamsters Infected with *B. microti*

ters gradually recovered afterwards, they were not completely restored until 24 weeks PI. Reticulocyte counts, showing the level of hyperfunction for hematopoietic organs of the hamsters, began to increase from 3 weeks PI (254.5/µl on Av) and peaked at 5 weeks PI (906.2/µl on Av) in response to parasitemia (Fig. 4D).

MCV (mean corpuscular volume) increased from 3 weeks PI and reached the peak at 4–6 weeks PI (77.9–82.8 fl on Av). The values gradually decreased from 8 weeks PI and restored the same level as in the control group at 13 weeks PI (55.4 fl on Av) (Fig. 5A). In contrast to MCV, MCHC (mean corpuscular hemoglobin concentration) decreased from 3 weeks PI and reached the bottom line at 5 weeks PI (18.6% on Av), but it restored the same level as in the control group at 9 weeks PI (26.6% on Av) (Fig. 5B). There were no differences in MCH value and TP (total protein) between the infected and control groups (data not shown).

Leucocyte count began to increase from 3 weeks PI and reached the peak at 4 weeks PI (118.6 × 10²/µl on Av). After that WBC count decreased and then restored the same level as of the control group at 7 weeks PI. However, no difference was recognized between the infected and control groups over 9 weeks PI. On the other hand, band neutrophil count began to increase at 2 weeks PI (174.5/µl on Av) and reached the peak at 3–4 weeks PI.

![Fluctuation of parasitemia in the blood of the hamsters infected with Djungarian hamster-adapted *Babesia microti*.](image)

**Fig. 3.** Fluctuation of parasitemia in the blood of the hamsters infected with Djungarian hamster-adapted *Babesia microti*. ○: Infection group.

![Fluctuation in RBC count (A), PCV (packed cell volume) (B), Hb (hemoglobin concentration) (C) and reticulocyte count (D) estimated in the blood of the hamsters infected with Djungarian hamster-adapted *Babesia microti*.](image)

**Fig. 4.** Fluctuation in RBC count (A), PCV (packed cell volume) (B), Hb (hemoglobin concentration) (C) and reticulocyte count (D) estimated in the blood of the hamsters infected with Djungarian hamster-adapted *Babesia microti*. ○: Infection group, ●: Control group.
Total and band neutrophil counts were higher than those of the control group from 2 to 8 weeks PI. In segment neutrophil count, no difference was recognized between the infected and control groups (data not shown). Lymphocyte and monocyte counts increased at 2 weeks PI and reached the peak at 3 weeks PI (736.1/µl on Av.) and 4 week PI (8181.2/µl on Av.) (Fig. 6D) and 4 week PI (8181.2/µl on Av.) (Fig. 6E), respectively. Lymphocyte count showed no difference between the infected and control groups until 24 weeks PI, but the monocyte count of the infected group was significantly high in comparison with that of the control group from 2 to 19 weeks PI. Eosinophil count increased at 2 weeks PI (480.4/µl on Av), but the hamsters developed eosinophilic leukopenia and the value was significantly low in comparison with that of the control groups from 4 to 24 weeks PI (Fig. 6F). Basophil count showed no difference between the infected and control groups (data not shown).

**DISCUSSION**

In this report, the susceptibility to the infection with *B. microti* was examined among the Djungarian, Syrian, and Chinese hamsters as well as the BALB/c mice based on the level of parasitemia, and consequently, Djungarian hamsters had the higher susceptibility than Syrian hamsters which are generally used for the isolation, maintenance and passage of *B. microti*.

In Exp 2, we examined the clinical signs and hematological characters of the Djungarian hamsters infected with the hamster-adapted *B. microti* strain until 24 weeks PI. Remarkable symptoms observed were hemoglobinuria clinically, anemia hematologically, and splenomegaly macroscopically, but neither cheerful elimination nor anorexia was observed throughout the observation period for 24 weeks PI. It is known that mice infected with *B. rodhaini* show hemoglobinuria and die within several days PI [13]. Bovine babesiosis by *B. bigemina* or *B. ovata* includes the signs of anemia, enervation, excretion of hemoglobinuria, and splenomegaly, but does not end in death [5, 22]. Canine babesiosis by *B. gibsoni* shows the sign of hemoglobinuria, but few infected dogs die [7]. Furthermore, in canine babesiosis a distinct anemia remains after the decline of parasitemia, in addition to these clinical signs [7]. In the present examination, the Djungarian hamsters infected with *B. microti* showed the clinical signs of anemia, hemoglobinuria, and splenomegaly, but not enervation. Parasitemia began to rise at 2 weeks and peaked at 4 weeks PI. After that, parasitemia declined gradually but maintained a level of about 10% on average until the end of the experiment (24 weeks PI). These findings suggest that Djungarian hamsters cannot completely exclude the parasites and might become a reservoir host through out their life, like cows and dogs infected with *Babesia* parasites do [14].

It was reported that parasitemia was more severe in mice infected with the mouse-adapted *B. microti* strain than in those infected with the non-adapted strain. Experiments 1 and 2 revealed that parasitemia in Djungarian hamsters until 10 weeks PI differed in severity when the animals were infected with different strains of *B. microti*, that is, it was more severe when infected with the Syrian hamster-adapted strain (Exp. 1) than with the Djungarian hamster-adapted strain (Exp. 2). The reason for this result was unclear.

In contrast with a rise in parasitemia, RBC count, Hb and PCV as anemic index fell to the lowest level at the 4 or 5 weeks PI. The Djungarian hamsters had macrocytic hypochromic anemia due to the increase of MCV and the decrease of MCHC in the growth phase of the parasite. After that, the values of parasitemia, MCV, and MCHC decreased, whereas reticulocyte RBC counts increased, and polychromatic erythrocytes appeared. These results indicate that myeloid function was enhanced and anemia was hemolytic and regenerative. Parasitemia gradually fell down the fourth week PI and the hamsters recovered from anemia. The results obtained strongly support the previous reports [5], in which anemia progresses synchronously with parasitemia. In addition, hemoglobinuria to excrete free-hemoglobin produced by the destruction of parasitized erythrocytes and splenomegaly due to the treatment of para-
sitized erythrocytes were clinically observed. These clinical signs suggest that the anemia in Djungarian hamsters infected with *B. microti* was mainly caused by the destruction of erythrocytes parasitized by the protozoan in the spleen.

Total WBC count increased and reached the peek level at 3 weeks PI. This increase was mainly due to the rise in immature granulocytes (band neutrophil), lymphocytes, and monocytes. However, in the Djungarian hamsters infected with *B. micorti*, total WBC count was scarcely different between the infected and control groups during the experiment. Only monocyte count was higher in infected group than in the control group during the course of experiment. It was reported that lymphocyte count increases in the spleenectomized cows infected with *B. bovis* and *B. bigemina* [22]. The high level of parasitemia in the Djungarian hamsters infected with *B. microti* in the chronic or convalescent stage of infection might be due to the inadequate numbers of

Fig. 6. Fluctuation in WBC count (A), total neutrophil count (B), band-neutrophil count (C), monocyte count (D), lymphocyte count (E), and eosinophil count (F) in the blood of the hamsters infected with Djungarian hamster-adapted *Babesia microti*. ◦: Infection group, □: Control group.
immature neutrophils and lymphocytes. Especially, an inadequate number of lymphocytes may inhibit the immunity specific to \textit{B. microti} produced by various kinds of lymphocytes, despite an increased number of monocytes as antigen-presenting cells. This may indicate high sensitivity of Djungarian hamsters to \textit{B. microti} infection.

Eosinophil count increased at 2 week PI and showed a transition at the lower level than that of noninfected hamsters throughout the experiment. This phenomenon, eosinophilic leukopenia, was not shown by Syrian and Chinese hamsters, and mice in this examination (data not shown). Eosinophilic leukopenia is generally due to over secretion of glucocorticoids in Cushing’s syndrome or stress with the decrease in lymphocyte count \cite{2}. However, the phenomenon in this study was not accompanied by the decrease in lymphocyte count, so we could not explain the phenomenon.

In Exp 1 and 2, in the Djungarian hamsters infected with \textit{B. microti} parasitemia rised from 2 weeks PI, and peaked at 4 weeks PI, after that it gradually decreased, but in Exp 2 parasitemia was at a level of about 10\% in 24th weeks PI and the parasites did not disappear in the peripheral blood. Since the Djungarian hamsters examined, are easily bred and kept with comfortable handling, and had highest sensitivity to \textit{B. microti} infection, so they would be useful for infective experiment, isolation, maintenance, and the passage of the parasite in laboratories.

REFERENCES