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# Immunization of Recombinant Membrane Protein in *Theileria sergenti*

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# *Theileria sergenti* 재조합 항원단백질의 면역원성

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**요 약 : Theileriosis에 대한 효율적인 예방대책을 마련하기 위한 일환으로 발현된 *T. sergenti* 재조합 항원단백질의 면역원성을 조사하였다. 먼저, *E. coli* 단백질 발현 vector인 pQE 32 plasmid vector를 이용하여 발현된 *T. sergenti*의 재조합 막표면단백질(KTs-MP)을 4개월령의 유우 송아지에 접종하였다. 그리고 접종된 송아지의 혈액변화상과 *T. sergenti*에 대한 항체가의 변화상을 분석한 결과, 재조합단백질의 접종에 의하여 항체가가 상승되는 것을 알 수 있었다. 그러나 재조합단백질의 접종만으로는 *T. sergenti*의 감염을 완전하게 예방하지는 못하였다.**

***Key words*** **:** *Theileria sergenti*, Recombinant protein, ELISA

## Introduction

Theileriosis, caused by *Theileria sergenti* infection, divided into a peracute, acute, subacute, mild or chronic forms[18]. A common and major clinical sign with all forms of bovine theileriosis is anemia, and last for 4~20 days[18]. For example, depression, hyperthermia, retarded growth, reduced milk production are due to a severe anemia. Also, theileriosis brought great economic loss in cattle of Korea[20].

Many studies indicate that anemia is introduced by the effects of opsonin with auto-immune responses against infected erythrocytes. Asaoka *et al.*[1] reported that macrophage are significantly activated within one month after inoculation, and these phenomenon appeared earlier than parasitaemia or the peak of antibody titer against *T. sergenti*[4,16]. Hagiwara *et al.* reported that a significant hemolytic activity is observed in the infected blood when parasitemia is in high phase[5,17]. But, the process of anemia is not clearly established yet.

Because of low mortality of mild or chronic theileriosis, many of veterinarian and farmer frequently ignore the significance of the consumptive disease. The individual treatment against theileriosis is require expensive veterinary and other charge, so the prevention or group management should be established for the control of theileriosis.

Research on the control of parasitic diseases through vaccination has been recently stimulated by the advance in immunology and biotechnology. In a general immunological aspect, the best way to prevent disease is immunization with a safe and effective vaccines. Hooshmand[8] reported that the hemoparasitic infection could be controlled by vaccination as in virus or bacteria infection. Also, several forms of live or killed vaccines were used to prevent theileriosis. But these vaccines were not effective to use in practice[8]. In this study, we evaluate the immunization effects of the purified recombinant *T. sergenti* membrane protein (KTs-MP) *in vivo*.

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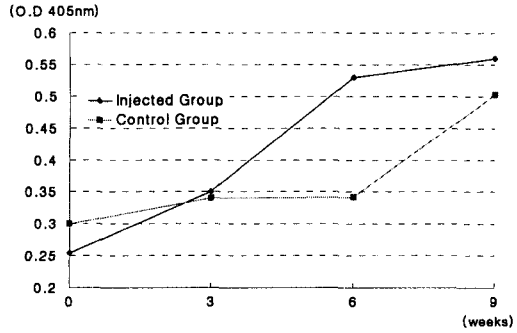


**Fig 1.** Levels of anti-*T. sergenti* antibody response after injection of recombinant KTs-MP protein.

## Materials and Methods

### Experimental Animal

The immunogenicity test were performed to evaluate the effect of recombinant KTs-MP *in vivo*. Four months of Holstein calves were used in this study. Among the healthy fifteen calves, ten were chosen as a experimental (injected) group, and five as a control (not-injected) group.

### Immunization

The purified recombinant KTs-MP were adjusted to 0.5 mg/m*l* according to the standard procedure[10] and injected into the experimental animals to evaluate the effects of recombinant protein immunization *in vivo*. Booster immunization was performed twice at successive 3 weeks interval following the initial injection. The experimental animals were immunized with 2 mg of recombinant KTs-MP protein in a total volume of 4 m*l*.

### Parameter of Evaluation

Prior to the immunization, all of the calves were checked with hematological values and antibody titer against *T. sergenti*.

### Hematological Examination

The whole blood and serum were collected from experimental and control calves. Erythrocyte count (RBC) and hemoglobin concentration (Hb) were checked by automatic blood analyzer (MINOS-VET, Japan), and Hematocrit (PCV) were measured by microhematocrit method. Hematograms of experimental group were compared with those of control group on the same times.

### ELISA

The level of KTs-MP specific antibody in calf's sera were measured by ELISA[13]. For ELISA, each wells of the microtiter plate were coated with 0.5 μg of isolated *T. sergenti* protein in 50 μ*l* of coating buffer, washed three times with PBS/0.05% Tween-20 and 200 μ*l* of blocked with blocking solution. Calves' sera serially diluted in PBS was added into well and anti-bovine IgG conjugated with AP was added onto each well. Absorbance was measured at 405 nm by using ELISA reader (Spectra Count, Packard instrument Co., USA). The antibody level of experimental group against *T. sergenti* was compared to control group on the same times.

## Results

### Hematological Values

Prior to the immunization, the mean values of PCV (%), Hb (g/d*l*) and RBC (×$10^6$/μ*l*) were 30.2, 10.6 and 5.5 in the experimental group, respectively. The same items were 33.2, 11.3 and 7.5 in the control group, respectively. These data showed the mean values of

**Table 1.** Hematological Values of Experimental (Injected with Recombinant KTs-MP) and Control Calves

| | | Experimental group (n=10) | Control group (n=5) |
|---|---|---|---|
| 0 weeks | PCV(%) | 30.2 | 33.2 |
| | Hb(g/d*l*) | 10.6 | 11.3 |
| | RBC(×$10^6$/μ*l*) | 5.5 | 7.5 |
| 3 weeks | PCV(%) | 28.1 (- 7%) | 29.8 (-11%) |
| | Hb(g/d*l*) | 8.7 (-18%) | 9.2 (-18%) |
| | RBC(×$10^6$/μ*l*) | 5.4 (- 3%) | 6.2 (-17%) |
| 6 weeks | PCV(%) | 24.3 (-20%) | 22.6 (-32%) |
| | Hb(g/d*l*) | 7.9 (-25%) | 7.2 (-36%) |
| | RBC(×$10^6$/μ*l*) | 4.4 (-21%) | 4.3 (-43%) |
| 9 weeks | PCV(%) | 25.6 (-15%) | 25.2 (-24%) |
| | Hb(g/d*l*) | 9.5 (-10%) | 9.5 (-16%) |
| | RBC(×$10^6$/μ*l*) | 6.3 (+11%) | 5.5 (-27%) |

( ); Decreased percentage compare with the preinjected data

PCV, Hb and RBC in control group were higher than experimental group

But six weeks later, the mean values of PCV, Hb and RBC in experimental group were higher than control group (Table 1). The mean values of PCV (%), Hb (g/dl), and RBC ( $\times 10^6/\mu l$) were 24.3, 7.9 and 4.4 in the experimental group, respectively. The same items were 22.6, 7.2 and 4.3 in the control group, respectively.

**Antibody Titer Against *T. sergenti***

The level of antibody titer was significantly increased by 6 weeks after innoculation in experimental group whereas the control group showed no significant change by 6 week (Fig 1).

## Discussion

Theileriosis is a protozoan disease that causing anemia, hyperthermia, reduced milk production, and severe economic loss of cattle industry in Korea[19]. If the cattle which previously infected with *T. sergenti* are exposed to strong stress or infected with other disease, they become severe anemia, icterus and pyrexia or death[21]. In the view of diagnosis, the diagnostic method progressed from the microscope examination to the molecular biological methods for the rapid and sensitive diagnosis. In molecular biological diagnostic methods, Southern hybridization using DNA probe, PCR amplification on the base sequenced target gene, phylogenic assay using 18s rRNA, Western blotting proved antigenic protein were continually developed[3,9,11].

The several effective prevention methods against theileriosis were reported. Hooshmand *et al.* reported that vaccination by tissue culture was effective for prevention of theileriosis[2,8]. However, owing to the difficulty of culture of *Theileria* spp., tissue culture vaccines are thought to be not feasible yet.

Most of the reports described about the effects of vaccination with recombinant protein to control the diseases including babesiosis and theileriosis[7,12,14]. Hines *et al.*[6] reported that vaccination with recombinant protein can produced high-antibody titer but does not reflect *in vivo* protective immunity to babesiosis. In this experiment, calves immunized with recombinant KTS-MP showed the increased antibody titer to *T. sergenti* compared with control calves. But immunization with recombinant protein could not perfectly prevent challenged with field *T. sergenti.*

## Conclusions

The purified recombinant antigenic protein of *T. sergenti* was evaluated for its immunogenicity *in vivo.* Holstein calves were immunized with the purified KTs-MP by 3 weeks interval. Then, the hematological values and the antibody titer against *T. sergenti* were checked from 0 to 9 weeks at successive 3 week intervals. The antibody titer of calves immunized with recombinant KTs-MP were increased significantly. But, the immunization with recombinant protein didn't protected perfectly challenged with field *T. sergenti.*

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