

Immunogenicity and Protective Efficacy of Solubilized Merozoite-enriched *Theileria sergenti* Immunogens. II: Protection against Natural Exposure under Field Conditions

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Abstract: A *Theileria sergenti* soluble merozoite preparation containing the 29, 34, 35 and 105 KD as the immunodominant polypeptides, was evaluated for efficacy, safety and protectivity in Holstein calves against virulent field tick challenge. The soluble antigens (100 mg/dose) were fortified with either complete or incomplete Freund's adjuvant. Twenty naive calves, aged one month, were subcutaneously inoculated with the preparation and a booster dose was administered 4 weeks later. Twenty additional calves served as controls. Five weeks after the booster dose, vaccinates and uninoculated controls were moved to a pasture, a heavily tick infested area in Cheju-do, Korea. The vaccinates showed negligible change in hematocrit and total RBC count whereas control animals showed significant ($p < 0.05$) hematological changes and associated anemia. Only 30% of vaccinates required chemotherapy after the experiment was terminated. All control animals required chemotherapy and 25% received blood transfusion. The highest percent parasitized erythrocytes in vaccinated cattle was 0.4% as compared with 3.6% among controls during the month of July. A significant difference ($p < 0.05$) was observed in the rate of body weight increase. Significant differences were also noted in serum albumin, aspartate aminotransferase, total protein and bilirubin. Significantly more vaccinated cattle maintained normal ranges of hematological and biochemical values as compared with the control group. It is suggested that soluble merozoite *T. sergenti* antigens may be potential vaccine candidates for developing a genetic vaccine in Korea.

Key words: *Theileria sergenti*, merozoite, vaccine, natural exposure, protection

INTRODUCTION

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Tropical theileriosis caused by *Theileria sergenti* (Chang, 1974; Han, 1971) is an important hemotropic disease in Korea and other parts of

Asia. Cardinal signs include progressive anemia, high morbidity and low mortality in native Korean cattle (Chun *et al.*, 1978) and a high mortality among imported temperate breeds (Park *et al.*, 1963; Suh, 1982). The major achievements on the characterization of the Korean isolate have been on morphological and immunological aspects (Baek *et al.*, 1990 a & b, 1991 & 1992; Kang *et al.*, 1988; Suh *et al.*, 1972). Recently, Tanaka *et al.* (1991) showed that anti-idiotypic antibodies against idiotypes of specific anti-*T. sergenti* antibody were immunogenic but not protective against virulent challenge. Other possible control measures include tick control, treatment of clinical cases, chemoprophylaxis and immunoprophylaxis. None of these methods either individually or collectively have been shown to be effective in the control of theileriosis. However, immunoprophylaxis appears to offer the best long term solution to this disease.

The most extensive immunological studies on theileriosis have focused on *T. parva* (Brown *et al.*, 1971; Burridge *et al.*, 1972; Dolen *et al.*, 1980; Mutugi *et al.*, 1991; Radley *et al.*, 1975 a & b; Sugimoto *et al.*, 1991) and Mukhebi *et al.* (1992) reported about the economic analysis due to theileriosis. However, very little research has been carried out on immunoprophylactic control of the disease caused by *T. sergenti* (Baek *et al.*, 1990a & 1991; Suh *et al.*, 1972) in Korea.

Recently, Baek *et al.* (1992) reported the first data on the development of a merozoite-derived preparation evaluated for antigenicity, immunogenicity, safety and protective efficacy against a homologous needle challenge with a virulent *T. sergenti* stabilate isolated from Korea. The latter report was under laboratory controlled conditions.

This follow-up report presents data on the field testing of the same immunogen for its efficacy and safety in protecting cattle against natural tick challenge under field conditions in Cheju Province, a heavily tick infested island.

MATERIALS AND METHODS

Isolation of *T. sergenti* and preparation of immunogen: The isolation of *T. sergenti* and preparation of immunogen was carried out basically as previously described (Baek *et al.*, 1990a & 1992). Briefly, *T. sergenti* was identified by light and electron microscopy and by immunologic techniques including western immunoblot (Baek *et al.*, 1990a & b). The infected erythrocytes were processed and stored in liquid nitrogen as described elsewhere (Love, 1972). Immunogen was prepared by a combination of sonication and differential centrifugation and characterized immunologically and physico-chemically.

Animals: All the animals used in this study were purebred Holstein calves aged 1 month. The calves were born and maintained on the same farm in Cheju Province, Korea. Prior to vaccination, the calves were kept indoors under a strictly tick-free environment throughout the months of December to February. The animals were fed optimal high protein diet and allowed *ad libitum* access to clean drinking water. There was no evidence of tick exposure either before or during the vaccination period. A significant number of these animals, however, were positive for *T. sergenti* parasites by Giemsa stain of thick blood smear. However their hematological and biochemical values were normal (Kaneko, 1989; Schalm *et al.*, 1975). Twenty animals were vaccinated and another 20 served as matched controls.

Vaccination and field challenge: Vaccinates received a primary dose of 100 mg (protein content: Lowry *et al.*, 1951)/animal fortified with complete Freund's adjuvant administered subcutaneously. One month later, vaccinates received a booster dose of 100 mg/animal again given subcutaneously. Controls received sham primary and booster doses at the same intervals. One month after vaccination, the animals were moved to an outdoor pasture heavily infested with ticks. These ticks (*Haemaphysalis longicornis*)

were known to have a high proportion of infection with *T. sergenti*(Kang *et al.*, 1989). Within a short period of time, ticks were observed to attach and feed on all the calves in the pasture.

Clinical, hematological and biochemical monitoring: All the animals were monitored at monthly intervals over a period of seven months(covering both spring and summer). The major parameters were overall clinical condition, body weight, hematocrit, total erythrocyte count and complete blood differential count. In addition, clinicobiochemical profiles (including renal and hepatic functions) were routinely monitored(Baek *et al.*, 1991. ; Kaneko, 1989; Scham *et al.*, 1975). Quantitative parasitological measurements were done on all animals as percent parasitized erythrocytes(PPE).

Serology: The serological test used was the indirect fluorescent antibody(IFA) test according to the procedure described by Baek *et al.* (1992). The IFA titers of 3 animals randomly selected from each group was determined chronologically on a monthly basis.

RESULTS

Monitoring of body weight: The comparison of body weight of vaccinated and control cattle is shown in Fig. 1. In general, all the animals in both groups, showed steady increase in weight

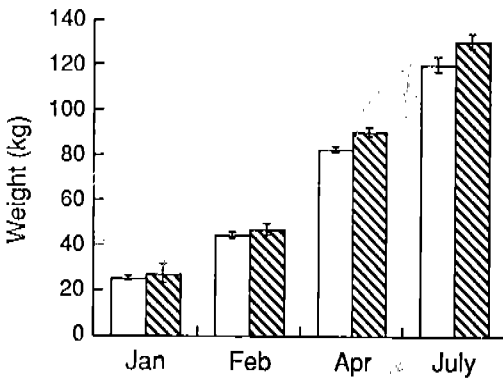


Fig. 1. Comparison of body weight(kg) between vaccinated(▨) and control(□) cattle on a monthly basis.

throughout the seven months of observation. There were no significant differences between the two groups during the months of January through June. In July, a significant difference ($p < 0.05$) was observed between controls(120.4 ± 3.30 kg) and vaccinates(130 ± 3.21 kg).

Parasitemia: The trend of increase in PPE among the two groups is presented in Fig. 2. *Theileria sergenti* parasites were detected in both groups of cattle prior to vaccination. Nearly 80% of all animals tested were negative to all hemoparasites while the remaining 20% were positive at approximately 0.03% PPE of *T. sergenti*. Significant differences ($p < 0.05$) of PPE between the two groups were observed from March through July. A highly significant difference was particularly evident during the month of July, with the vaccinates having PPE of less than 0.4% whereas the controls showed PPE of between 3.5% and 4%.

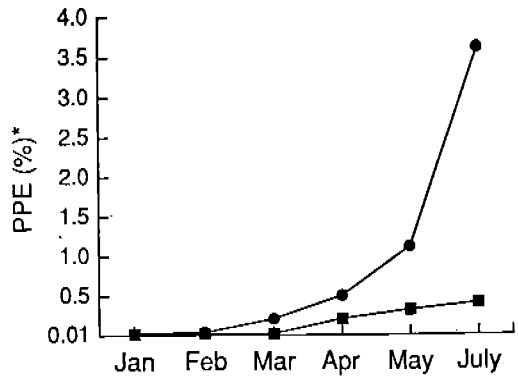


Fig. 2. Relative parasitemia(%) among vaccinated (-▣-) and control(-●-) cattle.

*PPE: Percent parasitized erythrocytes

Clinical signs and hematological values: The clinical condition of the control animals deteriorated so much that the experiment had to be terminated at seven months. The major complications were associated with a very severe hemolytic anemia. Significant drops in hematocrit and RBC were observed in both groups but vaccinated animals appeared to compensate effectively as indicated in Fig. 3. The biochemical profiles were generally unremarkable with the exception of significantly ($p < 0.05$) higher levels of bilirubin consistent with severe intra-

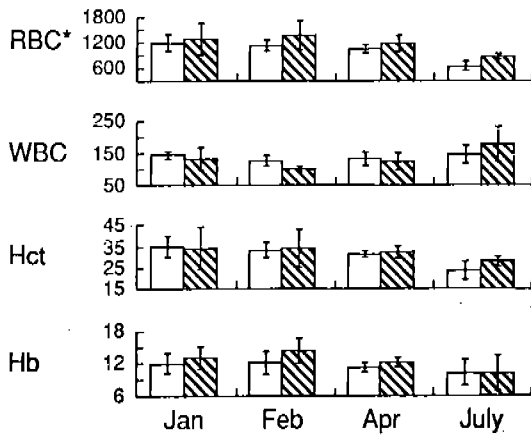


Fig. 3. Level of total erythrocytes, WBC, Hct, and Hb in vaccinated (▨) and control (□) cattle.
 *RBC: Red blood cell count($\times 10^6$), WBC: White blood cell count($\times 10^3$), Hct: Hematocrit(%), Hb: hemoglobin(g/dl)

vascular hemolysis in July as illustrated in Fig. 4. The levels of LDH in the control animals was moderately lower than in the vaccinated group. The values of total protein(Tp) and aspartate aminotransferase(AST) of both groups in July were found to be significantly($p < 0.05$) the highest of the levels observed throughout the entire experimental period and appeared to be due to theileriosis. During the same period, the level of bilirubin was, however, significantly higher in the controls as compared to the vaccinated animals.

Indirect fluorescence assay: With respect to IFA, no significant differences were observed in the overall trend of the IFA response. However, significant differences were observed between the two groups from 2 months post vaccination. Fig. 5 summarizes the reciprocal IFA titers of 3 animals randomly selected from each experimental group. The baseline titer was below 1 : 40. Following field tick challenge, the IFA titer of control animals rose to 1 : 1280 in July and that of the vaccinated group was boosted by natural infection to 1 : 2560.

Treatment: During the entire observation period, none of the animals in the two groups received treatment with either drugs and/or blood transfusion until the end of July when

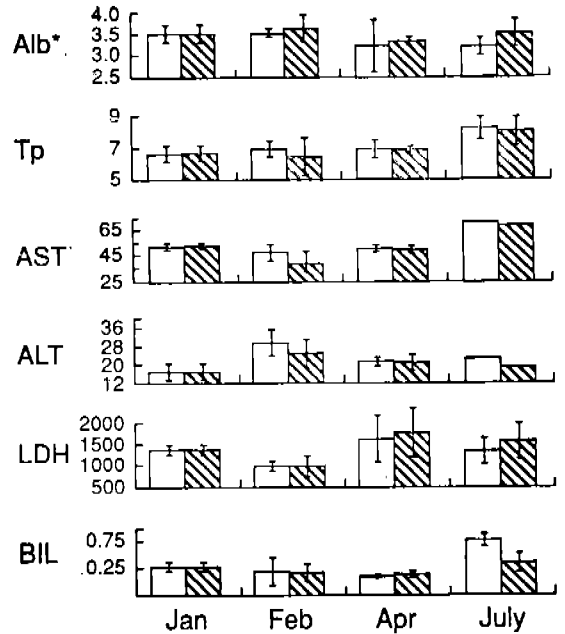


Fig. 4. Biochemical profiles of vaccinated(▨) and control(□) cattle.
 *Alb: Albumin(g/dl), Tp: Total protein(g/dl), AST: Aspartate aminotransferase(K.U.), ALT: Alanine aminotransferase(K.U.), LDH: Lactic dehydrogenases(K.U.), BIL: Bilirubin(mg/dl)

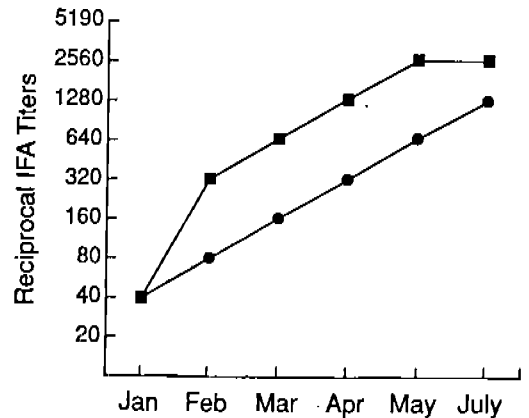


Fig. 5. Reciprocal IFA titers of vaccinated(-■-) and control(-●-) cattle.

the experiment was terminated due to severe anemia. The animals were treated by either a babesicidal drug (Berenil®) and/or by blood transfusion depending on the clinical status of each case. Six of the twenty controls received a transfusion at the end of July and all of control

cattle also were treated with Berenil®. Following intervention, all the animals survived. Only 20% of vaccinated cattle required treatment.

DISCUSSION

Theileriosis in Korea is economically one of the most serious tick borne protozoan parasitic diseases of grazing cattle. There is a need for the development of immunization methods to reduce the losses due to this economically important disease (Kim *et al.*, 1984) as proposed for *Theileria parva* infection. The conventional methods of control include chemoprophylaxis and treatment of clinical cases in addition to rigorous dipping protocols. These operations require expensive veterinary and other charges making them too costly and difficult to standardize. The annual increase in beef consumption in Korea has steadily increased over time necessitating a corresponding increase in beef imports from various countries. In general the mortality and morbidity of *T. sergenti* infection is much higher in exotic cattle than in native Korean animals (Park *et al.*, 1963). Suh *et al.* (1972) attempted to develop an attenuated whole blood vaccine against *T. sergenti*. There are a number of constraints associated with the use of this method of vaccination (Curnow, 1973; Dimmock *et al.*, 1970). Accordingly, we decided to investigate an alternative procedure of immunoprophylaxis utilizing relatively purified immunogens and optimal dosage and adjuvant regimen as previously discussed (Baek *et al.*, 1991).

The data presented above has shown for the first time that semipurified-merozoite enriched preparations of *T. sergenti* can confer significant protection in naive, highly susceptible, native Korean Holstein calves. More importantly, the protection was demonstrated under stringent, high transmission field conditions in a hyperendemic area of the disease. Our data showed that the best indices of protection were: anemia, a significant IFA response, improved body weight gain and moderate to low PPE. Although vaccinated calves were successfully infected by

tick challenge, they were able to control the parasites and return to normal hematocrit values at peak parasitemia. It is noteworthy that unvaccinated animals showed severe intravascular hemolysis and associated anemia and elevated blood bilirubin levels. The latter change coincided with a high PPE of 4%, low total erythrocyte counts and hematocrit, associated with significantly less gain in body weight. Further, there was an increase in the level of AST, ALT and bilirubin indicative of glomerular and hepatic malfunction.

The mechanisms of protection against *T. sergenti* infection have been reviewed extensively by many authors (Ohgitan *et al.*, 1987; Asaoka *et al.*, 1991; Yasutomi *et al.*, 1991). In Korea, a significant proportion of calves are infected with *T. sergenti* transplacentally (Baek, unpublished data). Approximately 20% of newborn calves in Cheju area show evidence of PPE and/or serological evidence of *in utero* exposure. Similar observations have been reported with respect to human malaria (Thompson *et al.*, 1977; Thomas *et al.*, 1982). In the current experiment, many of the calves used were parasitemic at birth. They were randomly assigned to the experimental groups. It is interesting to note that all calves, regardless of *in utero* infection, seroconverted following vaccination or simply natural infection via the tick vector. Thus, *T. sergenti* infection does not appear to induce measurable immunosuppression/tolerance after the fetus has been exposed transplacentally. In all, significant changes in antibody titers were observed between the two groups, both with and without, at weeks 1, 2 and 5 postexposure. Clearly, however, antibody levels *per se*, do not appear to correlate with protection since the unvaccinated group also showed significant seroconversion coinciding with the severely deteriorating clinical condition. However, it has been shown that cell-mediated immunity in concert with humoral response, may play a significant role in protection against this disease (Yasutomi *et al.*, 1991; Chaudhri *et al.*, 1992).

These preliminary data confirm our previous controlled laboratory experiments in which we demonstrated significant protection against artificial needle challenge (Baek *et al.*, 1992). It is necessary to point out that there are some difficulties involved in the purification of sufficient quantities of this antigen and that there may be unrecognized delayed or latent side-effects. We suggest, however, that our data provide a foundation for developing synthetic or recombinant vaccines that express the immunodominant epitopes (Baek *et al.*, 1992) which have been described for malaria (Amador, *et al.*, 1992; Patarroyo *et al.*, 1992; Rocha *et al.*, 1992).

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Theileria sergenti merozoite 수용성 항원의 항원성과 면역성 II. 자연 조건하에서의 감염에 대한 면역시험

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Theileria sergenti merozoite의 면역원성 polypeptide 중 29, 34, 35 그리고 105 KD를 함유한, 수용성 항원을 홀스타인 송아지(1개월령)에게 접종한 후, 제주도의 야외 목장에 방목시킴으로써 진드기로부터 병원성 총주의 도전에 따른 항원의 효력, 안전성 그리고 방어력을 관찰하였다. 즉 송아지 20두에 수용성 항원(100 mg/dose)을 Freund's adjuvant와 함께 1차 접종, 4주 후에 추가접종하였으며, 다른 20두의 송아지를 대조군으로 사용하였다. 추가접종 5주 후에 이들 송아지를 야외 목장에 방목, 매월 혈액학적 그리고 생화학적 변화를 관찰하였다. 예방접종군에 있어서 hematocrit와 총적혈구수는 진드기 도전 전과 비교하여 약간의 변화를 인정할 수 있었다. 일반적인 혈액학적 소견은 예방접종군과 비교하여 유의한 차이를 인정할 수 있었다($p < 0.05$). 7월에 있어서 적혈구내 기생충은 예방접종군에 있어서는 0.4%이었으나, 대조군에 있어서는 약 3.6%이었다. 체중 증가율, albumin, aspartate aminotransferase, total protein, 그리고 bilirubin에 있어서도 유의한 차이($p < 0.05$)를 인정할 수 있었다. 예방접종군의 혈액학적 그리고 생화학적 측정치는 대조군 보다는 정상치에 접근하였다. 실험종료 직후에 있어서 예방접종군의 30% 송아지와 대조군의 모든 송아지의 치료와 대조군 중 25%는 수혈이 요구되었다. *T. sergenti* merozoite의 수용성 항원에 대한 이 같은 연구 결과는 앞으로 유전공학적 백신제조를 위한 연구자료로 활용될 수 있을 것이다. [기생충학잡지, 30(3):201-208, 1992년 9월]