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Congenital transmission of *Theileria sergenti* in cattle verified by immunohistochemistry

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소 *Theileria sergenti*의 태반감염에 대한 면역세포화학적 증명

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초 록 : 소 theileriosis의 태반감염 사실을 입증하기 위하여 *Theileria sergenti*에 자연감염된 암소의 비장, 태반 그리고 유산된 태자의 비장과 태반조직으로부터 *T sergenti*를 면역화학적으로 검색 입증하고자 *T sergenti* 표피의 34KD 항원의 단크론 항체를 활용하여 avidin biotin complex 방법으로 면역화학적으로 염색하였던 바, 이들 formalin 고정 조직표본에서 *T sergenti*의 특이 항원성 물질을 관찰함으로써 태반감염을 증명할 수 있었다.

Key words : *Theileria sergenti*, congenital transmission, avidin-biotin complex(ABC) method.

Introduction

Theileriosis due to *Theileria sergenti* is considered to be one of the economically most important tick-borne protozoan disease of the grazing cattle in Korea[1]. The disease is characterized by inappetence, gradual weight loss, mild hyperthermia and anemia. The infected cattle when stressed with co-infection with other organisms, shipping show severe signs and sometimes die[2]. It has generally been established that theileriosis is transmitted through the vector ticks from the infected animals to a susceptible one(ie horizontal transmission). However, some haemotropic parasites like *Plasmodium falciparum* in human beings[3] and *Anaplasma* spp. in cattle[4] are known to be transmitted vertically/congenitally. Recently, we have reported vertical transmission of *Theileria* spp. in cattle[5], in which we verified the vertical transmission by detecting the parasite in

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blood smears stained with Giemsa and acridine orange, which are widely and routinely used as clinical approach for detecting the piroplasm stage of pathogenic *Theileria* spp. as well as other hemoprotozoa in erythrocytes. However, it is very difficult to distinguish the schizont stage in specimens from infected spleens by Giemsa's stain, because only a few organisms are present in the node and their extracellular location further complicates their detection from the smear preparations used for a routine examination[6].

In the recent years, immunohistochemical staining technique has been evaluated as one of the most sensitive and potential diagnostic tool to detect specific antigen[7], microoganisms[8], hormones[9] and tumor cells[10].

This technique has also been used for detecting the schizont stage of *Theileria* spp. in the infected lymph nodes[11]. In the present study, we have verified the congenital infection of *T sergenti* by a highly sensitive immunohisto-chemical technique, avidin-botin complex using *T sergenti* antigens with the monoclonal antibody encoding to the 34KD surface protein of *T sergenti*. One of the major goal of this study is to identify the parasite antigens which are localized in the fetal tissues and specific for the infectious form of the parasite, transmitted through the maternal blood(ie transplacental/congenital transmission).

Materials and Methods

Tissues of aborted fetuses and dams : The spleens and placentas from the fetuses which were aborted on 5th, 6th and 8th month of gestation were collected from Korean indigenous cattle naturally infected with *T sergenti* by direct microscopic examination of Giemsa stained blood smear. The placentas and spleens were also collected from the corresponding dams.

Paraffin section : The specimens were fixed in 10% neutral buffered formalin for 2 to 5 days. The fixed specimens were then processed in a tissue processor overnight, embedded in paraffin(avoiding heat over 60℃ and sectioned at 5μm thickness). The paraffin sections were placed on microslides coated with polylysine.

Pretreatment of the sections before immunostaining : Paraffin sections were deparaffinized and dehydrated by consecutive submersions in xylene(three changes, 3 minutes each), absolute, 95% and 70% ethanol(3 minutes each), and distilled water(3 minutes) following a routine histological procedures. Sections were counter stained with hematoxylin and eosin Y according to Thompson *et al*[12] and Tuan *et al*[13].

Avidine Biotin Complex Method(ABC method) : The tissue sections were digested with 1% trypsin in tris-buffered saline(TBS) for 30 minutes and treated with 3% hydrogen peroxide in methanol for 10 minutes at room temperature followed by several washes in TBS. The sections which were exposed to nonspecific antibody binding was blocked by 10-minute incubation at room temperature with 10% bovine serum albumin. The sections were incubated with monoclonal antibody[14] for 34KD surface protein of *T sergenti* diluted 1 : 100 at room temperature for 30 min in a moist chamber. The sections were then treated with biotin-labeled goat antimouse IgG antibody diluted as 1 : 200 followed by avidin-biotin-peroxidase complex kit(Dako Co.) The sections were treated with the chromogen 3-amino-9-ethylcarbazole to visualize immunoreactive parasites and then counterstained with hematoxylin[8,9,15]. As negative controls, the spleens and placenta sections were treated with normal rabbit and mouse sera at similar concentrations instead of monoclonal antibody and biotin-labeled anti mouse IgG according to ABC mothod[8,9].

Results

Routine histological findings : The parasites were not detectd from tissue sections of spleens and placenta obtained from the aborted fetuses as well as from the corresponding dams naturally infected with *T sergenti* stained with hematoxylin and eosin.

Immunohistochemical findings : The immunoreaction in the spleens and placenta were manifested as a totally distinct reddish colour using monoclonal antibody in the placenta of dams(Fig 1). The infected cells in placental tissues of the aborted fetuses stained pale red colour around the nucleus of the cell(Fig 2) and noninfected cell was not reddish colour. The staining reaction was typically of moderate to

strong in intensity with granular and diffuse cytoplasm in spleens. The schizonts in the cells were visualized as red colour by the ABC method using monoclonal antibody which could easily be distinguished from the uninfected cells in the spleens sections. The infected lymphocytes which stained the reddish colour(Fig 3) were clearly seen in the maternal spleen of the dam naturally infected with *T sergenti* and showed a scarcely infected cells with parasites (Fig 4).

However, it was not possible to see the internal structures of the schizont in ABC method under 1,250 and 1,000 magnification(Fig 3) in the dam's organ as well as fetus's organ.

The parasites were not detected in ABC stained tissues sections. Those were treated only with nonimmune serum and unconjugated antibody(negative control).

Discussion

Bovine theileriosis caused by *T sergenti* is one of the major source of economic losses in grazing cattle in Japan and Korea[1,16]. The disease has been diagnosed routinely on a clinical basis by direct microscopic examination of Giemsa-stained blood smear for piroplasm stage of the parasite. The exoerythrocytic forms of *T sergenti* can also be demonstrated in lymphnode of experimentally infected calves[6]. However, it is very difficult to distinguish the schizonts in specimens from infected lymphnodes by Giemsa's stain, because of only a few organisms which are present in the node and their extracellular location further complicates their detection from the smear preparations used for a routine examination[6]. however *T sergenti* in two countries might be difference molecularly. The monoclonal antibody was useful to identify *T sergenti* in Japan[14,17,18a].

In the present study, schizonts were detected in spleen and placenta of the aborted fetus as well as in the tissues (maternal spleen and placenta) of the corresponding dam naturally infected with *T sergenti* by the ABC method using monoclonal antibody encoded to the 34KD surface protein of *T sergenti*. The immunoreactive cells in fetal lymphnode were manifested as distinct red(Fig 4). The schizonts in the cells were visualized as red color which could easily be distinguished from the uninfected cells in the fetus placenta and lymphnode. The fetal placenta revealed a weak pale red colour around the nucleus of the cells(Fig 2) and scarcely infected cells with the parasites(Fig 4). The schizonts in the lymphocytes of the maternal spleen were also clearly seen (Fig 3). The structural characteristic of the schizonts were the same as those in Giemsa and acridine orange stained smears[5,11]. This findings suggested that *T sergenti* could be transmitted transplacently from the dams naturally infected with the parasite also confirmed our previous report[5] in which we proved the congenital transmission of *T sergenti* by detecting the parasites in Giemsa and acridine orange stained blood smears. *Theileria sergenti* is similar to many other protozoan parasite in having a complex multi-stage life cycle which involves two hosts. Namely, cattle and a tick vector possesses genomic diversity comparable to that of *Plasmodium* spp[3,19]. We have demonstrated that the 34KD surface protein of *T sergenti* targeted with the specific monoclonal antibody, facilitates the study of congenital infection with *T sergenti*.

It is hypothesized that the aborted fetuses might have been already infected with *T sergenti in utero*. Through immunohistochemical staining method, we have been able to demonstrate unequivocally that congenital transmission had occurred using monoclone antibody for 34KD surface antigen of *T sergenti*.

Summary

The spleen and placenta from the aborted fetuses as well as lymphnodes and placenta of the corresponding dams naturally infected with *T sergenti* were used to localize the parasite antigens by immunohistochemical staining for the possible congenital transmission of theileriosis. Parasite-specific antigens were detected immunohistochemically by incubating the sections with specific monoclonal antibody prepared against 34KD surface antigen of *T sergenti* and visualized via the avidin biotin complex(ABC) method. Specific *T sergenti* antigen was detected in the sections of formalin or acetone-fixed fetal spleens and placenta. Similar antigens

were also demonstrated in lymphnodes and placentas of the corresponding dams. It is concluded that this technique will eventually play an important role in specialized diagnostic laboratories in the verification/evaluation of congenital infection with *T sergenti*.

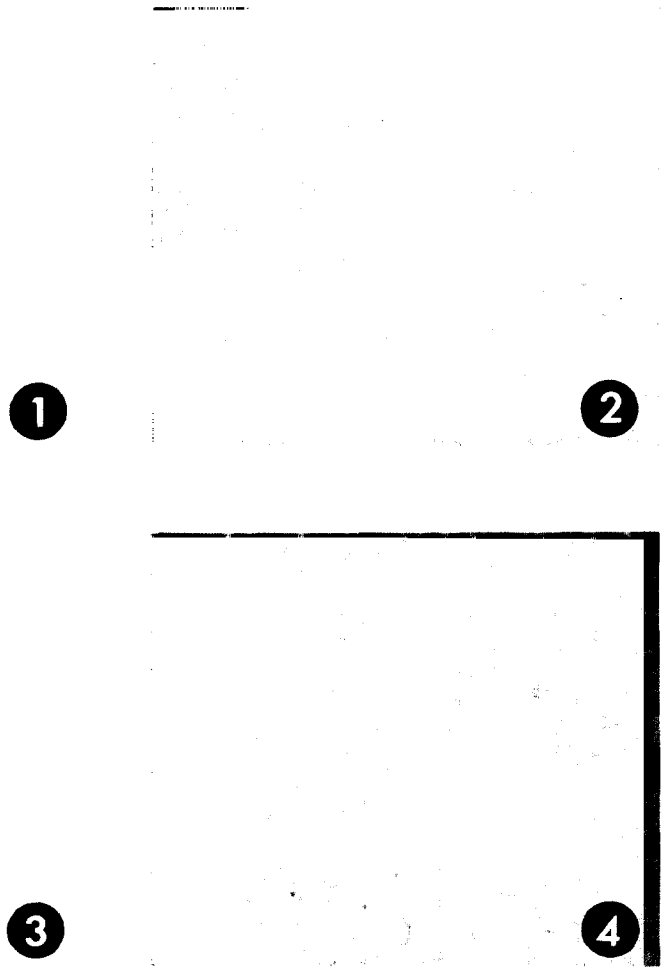
Legenders for figures

Fig 1. *Theileria sergenti* schzont stained by ABC method with hematoxylin counterstain was clearly differentiated as red color in placenta of *T seregnti* infected dam(X 1,000).

Fig 2. *Theileria sergenti* schzont stained by only ABC method was clearly differentiated as red color in aborted fetal placenta from *T seregnti* infected dam(X 1,000).

Fig 3. *Theileria sergenti* schzont stained by only ABC method was clearly differentiated as red color in aborted fetal spleen from *T seregnti* infected dam(X 1,000).

Fig 4. *Theileria sergenti* schzont stained by ABC method with hematoxylin counterstain was clearly differentiated as red color in aborted fetal spleen from *T seregnti* infected dam(X 1,000).



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