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Scanning electron microscopy on proliferative forms of *Toxoplasma gondii* and *Sarcocystis* species

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Toxoplasma gondii 와 *Sarcocystis* 原蟲의 增殖型에 對한 走査電子顯微鏡的 觀察

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抄 錄 : 哺乳類의 主要 寄生原虫인 *Toxoplasma gondii* 와 *Sarcocystis* species의 增殖型에 對한 表面微細構造를 比較하기 위하여 走査電子顯微鏡으로 觀察하였다.

同 試料는 人工感染된 마우스로부터 分離된 *T gondii* 와 自然感染된 屠畜牛로부터 分離된 *Sarcocystis* species의 增殖型이었다.

觀察結果 마우스 腹腔으로부터 採取된 *T gondii* 의 增殖型인 tachyzoites는 초승달모양(crescent-like feature)을 나타냈고, 計測結果 길이 5.57μm, 폭 2.33μm 이었으며 屠畜牛의 心筋에서 採取된 *Sarcocystis* species의 增殖型인 bradyzoites는 바나나모양으로 길이 14.18μm, 폭 2.85μm 이었다.

特히 *Sarcocystis* species 增殖型의 表面觀察에 있어서 높은 擴大倍率(60,000X)의 觀察計測 結果, 길이 0.35μm, 폭 0.18μm 의 微細孔(micropore)이 確認되었다.

Key words: *Toxoplasma gondii*, *Sarcocystis* species, SEM, proliferative form, mice, cattle.

Introduction

The Genera *Toxoplasma* and *Sarcocystis*, and related protozoa belonging to the Family Sarcocystidae, the Order Eimerina, are now recognized as important parasites not only in small animals[1] but also in cattle.[2~7] The diagnosis for *Toxoplasma* and *Sarcocystis* infections has usually been done morphologically or immunologically.

In immunological diagnosis, cross reaction between *Toxoplasma* and *Sarcocystis* in various serological tests has generally been believed not to occur, although Barriga[8] has reviewed that some cross-reactivity has been found between the species with the indirect fluorescent antibody(IFA) test, but not with the methylene blue dye(MBD) nor the indirect haemagglutination(IHA) tests, and that different species of the Genus *Sarcocystis* possess common antigens and antigens peculiar to each species[9]. Uggla et al[10] have reported that the question of the role of *Sarcocystis cruzi* and possibly other *Sarcocystis* infections when recording low-positive *Toxoplasma* antibody levels in cattle has been raised.

In morphological diagnosis, the light microscopy

has been used to detect the parasites of various stages in the host animals or in the intermediate hosts. However, the electron microscopy using transmission electron microscope (TEM) or scanning electron microscope(SEM) has been valuable in Toxoplasma studies with the informations based upon ultra-fine structures.[11,12] In this manuscript, the morphological comparison for the proliferative forms of *Toxoplasma* and *Sarcocystis* obtained by the scanning electron microscopy is reported with some SEM photographs.

Materials and Methods

Tachyzoites of *Toxoplasma gondii*: *Toxoplasma gondii* RH strain maintained in serial passages using mice in the Veterinary Research Institute, was used in this observation. The strain is highly virulent causing an acute fulminating and fatal disease in mice. The tachyzoites were collected from the peritoneal exudates of the mice on 4th (days after infection) DAI just before the experimental hosts die.

Bradyzoites of *Sarcocystis* Species: The bradyzoite forms of *Sarcocystis* species were collected from the heart muscle of the slaughtered cattle in an abattoir in Seoul. A piece of heart muscle was macerated and digested for one to two hours at room temperature in artificial digestion solution containing 0.5% trypsin formulation. After digestion, the digestate was filtered through a copper sieve and double-folded gauze into a beaker and allowed to sediment for an hour, after which small amount of the sediment was transfered to a slide-glass for the light microscopic observation[13] and to a micro-slide glass for the SEM preparation[6].

Preparation for SEM: The protozoan specimens were firstly fixed in heated 10% buffered formalin and refixed in 5% glutaraldehyde buffered with 0.1M cacodylate. The specimens were then smeared onto a micro-slide glass attached on the aluminium stub with silver paste. Eventually the specimens were dehydrated in graded ethanol series with three changes of absolute ethanol and finally with 100% acetone. The dehydrated specimens were then applied into an ion-coater(IB-3) using rotary vacuum evaporator for coating with gold (Au) ions.

Observation and measurement by SEM: The specimens were examined by a scanning electron microscope(Hitachi S-570). The observations were performed at 20 KV and with a magnifications ranges 1,000X to 6,000X. SEM photographs were recorded on polaroid Type 55 films.

Results

In Figs 1 and 2, the tachyzoites, a proliferative form of *T gondii* isolated from the peritoneal cavity of the mouse infected artificially was crescent-like features. Though a number of workers usually cites the term 'crescent-like' in the literatures, the feature in fact should rather be described as 'pine-rice cake shape' in Korean traditional concept. The tachyzoites of *T gondii* measured as shown in Table 1.

In Figs 3 and 4, the bradyzoites, a proliferative form of *Sarcocystis* species isolated from the heart muscle of the slaughtered cattle infected naturally was banana-shaped. As the feature appears an elongated and curved body, round bottom and conoid apex, it should rather be described as 'horn-like' in author's own opinion. The bradyzoites of *Sarcocystis* species measured as shown in Table 1.

By light microscope, a micropore is seen as a dot on the surface of *Sarcocystis* body. The micropore was also observed by scanning electron microscope as a distinct elliptical feature, and it measured as 0.35

Table 1. Comparison of the body size in the proliferative forms of *Toxoplasma gondii* and *Sarcocystis* species

Species	Developmental stage	Length(μm)	Width(μm)	Ratio(L/W)
Toxoplasma gondii	Tachyzoites	3.23~6.18 (5.57±0.86)	2.07~2.89 (2.33±0.12)	2.39*
Sarcocystis Species	Bradyzoites	12.06~16.25 (14.18± 1.14)	2.44~3.27 (2.85±0.36)	4.97**

* : crescent-like (pine-rice cake shape).
** : banana shape (horn-like).

μm in length and 0.18μm in width, and the ratio of length/width was 1.94 as shown in Fig 8 with high magnification of 60,000X. Figs 5 to 7 presented various sites of the micropores.

Discussion

As *Toxoplasma gondii* is common throughout the world, toxoplasmosis has been found in prctically several types of animals and about 25 to 30% of the human population of the world has antibodies against *Toxoplasma* antigen[14]. In the Genus *Toxoplasma*, merogony occurs in both the intermediate hosts, many types of animals including human beings, and the definitive hosts, members of the canivore Family Felidae[15]. The oocysts of *T gondii* are produced in the epithelial cells of the small intestine of the definitive host and are unsporulated when passed in the feces.[16] By ingesting sporulated oocysts or infected meat or animals, or congenitally via the placenta, the intermediate hosts become infected.[17] When the sporulated oocysts are ingested, the sporozoites emerge and multiply by endodyogeny in the cells invaded. This has been called a pseudocyst stage, and the merozoites within the pseudocyst are proliferative forms called tachyzoites. *Toxoplasma* tachyzoites are usually found in the leukocytes in peritoneal exudate or in other parenteral organs, and an acute toxoplasmosis occurs in this stage.[18,19] Meanwhile, a chronic toxoplasmosis occurs with bradyzoites formed by endodyogeny in a pseudocyst called meront, occurring commonly in the brain or in other tissues such as muscle.[14] However, there are no metrocyte stage in *T gondii* development compared with the metrocyte stage in *Sarcocystis*. The asexual stages of *Sarcocystis* occur in a prey animal, the sexual stages in a predator.[20,21] The definitive host becomes infected by ingesting fully matured *Sarcocystis* containing bradyzoite stage, usually by eating the prey animal. But the metrocytes are not infective for the predator host. The intermediate host can be infected by ingesting sporocysts, and there appear to be one or two generations of meronts in various organs, followed by another and final generation in the striated muscles. Tachyzoites are produced in a variable number and form the last meront generation called sarcocyst. It forms metrocytes and repeats endodyogeny to form intermediate stages and then bradyzoites.[22]

There are three species of *Sarcocystis* which infect cattle as an intermediate host. Heydorn et al have already proposed a new nomenclature of the sacosporidia in cattle as *S cruzi*(Syn *S bovicanis*), *S hirsuta*(Syn *S bovifelis*) and *S hominis*(Syn *S bovihominis*). *S cruzi* is common throughout the world. This species is not pathogenic for the definitive host dog but it is highly pathogenic for the intermediate host cattle, causing anemia, anorexia, cachexia, weight loss, abortion and death, called Dalmeny disease. *S hirsuta* is also common and is not or only slightly pathogenic for calves and apparently not at all pathogenic for cats. *S hominis* is also common and is slightly if at all pathogenic for calves and may cause diarrhea in man. However, in this presentaion, the species differentiation of *Sarcocystis* was not performed, the morphological finding for the comparison with *Toxoplasma* were only described on the base of SEM observations. It is hoped that the species identification of *Sarcocystis* in cattle in Korea would be clarified out through the artificial infection experiments to dogs and cats hereafter.

Through asymptomatic toxoplasmosis is the commonest type, toxoplasmosis may vary from an inapparent infection to an acutely fatal one. Levine[14] has mentioned that the most certain method of diagnosis for toxoplasmosis is by isolation of the parasites themselves by inoculation of experimental animals such as mice and rats, etc, and in this case *T gondii* can be found in smears of exudates, usually from the peritoneal cavity, as a proliferative form tachyzoites. However, it must be differentiated from other organisms, including *Sarcocystis*' which is not always possible on structural ground alone.

Some observations based on the transmission electron microscopy(TEM) of the morphology of *T gondii* have already been reported,[11,12,24~26] meanwhile, the author has been tried to apply a scanning electron microscope(SEM) for the observation of surface structures of the parasites.

Summary

For the comparison of surface fine structures in the proliferative forms of two major protozoan parasites, *Toxoplasma gondii* and *Sarcocystis* species in mammalian hosts, isolated from the artificially infected mice and from the naturally infected cattle, respectively, an SEM(Hitachi S-570) was applied to the fixed, dried and coated with gold ion on the microslide glasses.

The tachyzoites of *T gondii* from the peritoneal cavity of the mouse showed the crescent-like feature and measured as 5.57 μm in length and 2.33 μm in width, while the bradyzoites of *Sarcocystis* species from the heart muscle of slaughtered cattle was banana-shaped and measured as 14.18μm in length and 2.85μm in width. On the surface of *Sarcocystis* species bradyzoite, a distinct elliptical micropore was identified in the high magnification observation of 60,000X, and it measured as 0.35 μm in length and 0.13μm in width.

Legends for figures

*Scanning electron microscopy; Hitachi S-570, 20KV

Fig 1. Tachyzoites of *Toxoplasma gondii* isolated from the peritoneal cavity of the mouse infected artificially (1,000 X).

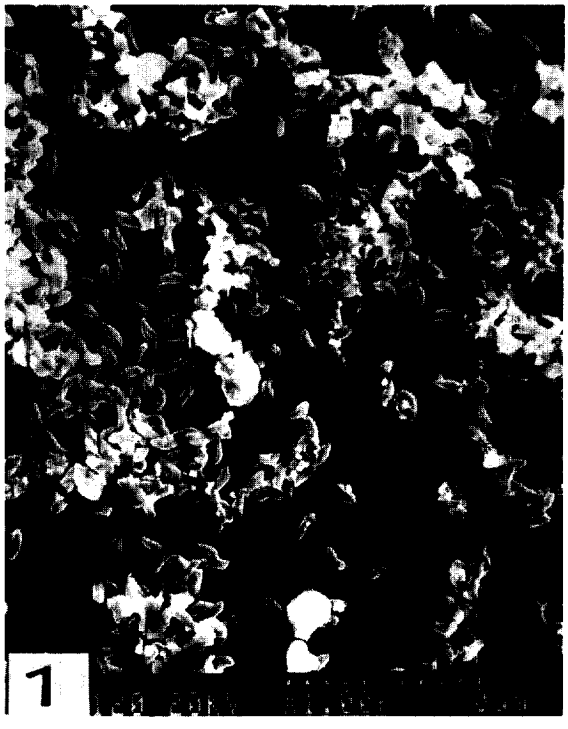
Fig 2. Crescent-like feature of *T gondii* tachyzoites(3,000X lower part and 15,000 X upper part).

Fig 3. Bradyzoites of *Sarcocystis* species isolated from the heart muscle of the slaughtered cattle infected naturally(4,000 X).

Fig 4. Banana-shaped *Sarcocystis* species bradyzoite showing elongated and curved body, round bottom and conoid apex(5,000 X).

Fig 5. to 7. Micropores(marked with arrows) on the body surface of *Sarcocystis* species bradyzoite(8,000 X in Figs 5 and 6 and 9,900X in Fig 7).

Fig 8. Micropore(in white square mark) and in ultsa-high magnification (6,000X lower part and 6,000 X upper part).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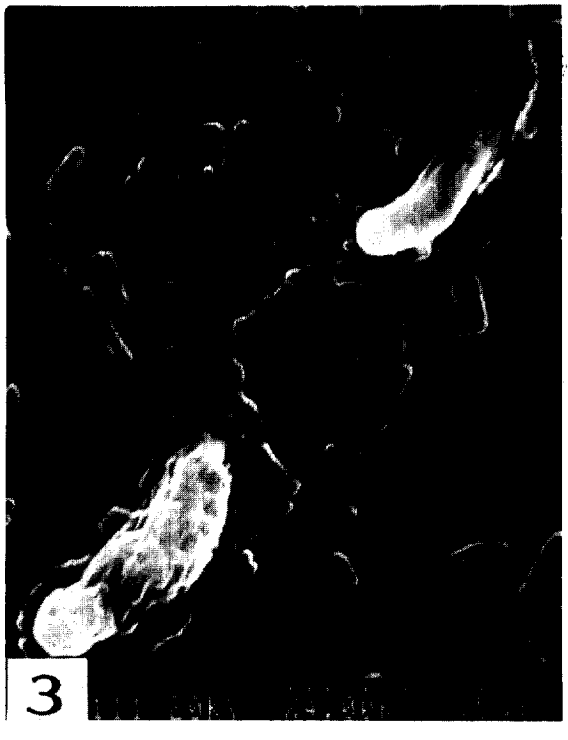
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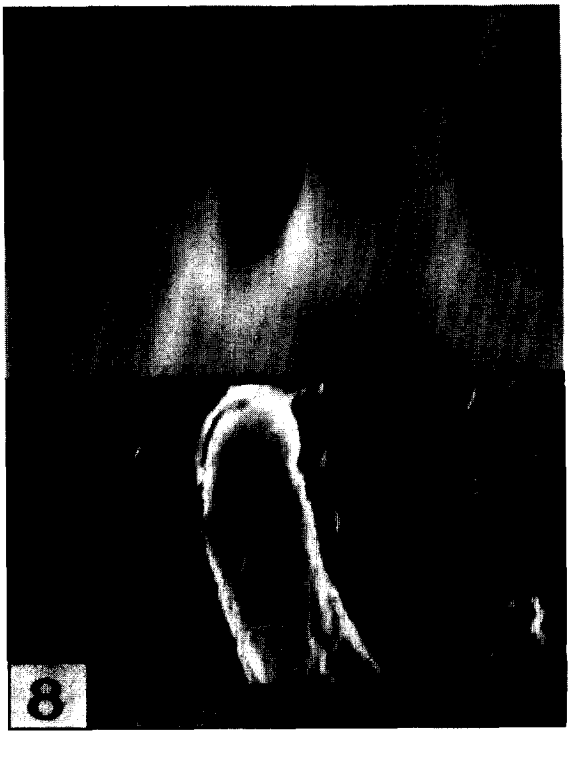
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