A STUDY OF SARCOSPORIDIA IN KOREAN CATTLE

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I. INTRODUCTION

Sarcosporidium is widely known as a protozoan parasite, found most frequently in meat animals and rarely in human being; up to the present more than ten cases as to the latter have been reported in foreign countries. Since the time when Miescher (1843) had found the whitish gray cylindrical body in the muscle fibres of mice, it has passed 85 years, during the course of which nearly one hundred authors have reported about it in many countries. Notwithstanding those unparalleled efforts made by them, the true nature of the microparasite is still so far from being clear. It may be reasonable to say that it is the privilege of the veterinary medical scientists to study this parasite because they have always at hand good rich of fresh materials and that the study of animal sarcosporidian parasite will be of much interest not only from the sanitary point of view but also from the point of view of parasitological research.

K. Yokota (1900) and S. Sato (1921) of Formosa in the water buffalo, I. Otsuka & Y. Kawasaki (1913) of Japan proper in a few carcasses of horses and cattle, S. Nakanishi (1926) of Fusan, in many Korean cattle, studied this parasite. T. Ohira (1911, 1912), a physician, investigated the nature of Sarcocystis muris, S. Sato (1926) studied the property of desiccated sarcosporidiotoxin prepared from Sarcocystis Blanchardi Doflein, and S. Taguma (1926) preliminarily reported his finding of the globidium-like nodules in the
abomasum membranes of sheep.

In our laboratory, we autopsied a large number of Korean cattle which were used for the production of anti-rinderpest serum and rinderpest vaccine. In a previous report we briefly described the morphology of bovine sarcosporocystis. Since, in the last successive four years we had made much efforts to the further examination of sarcosporidiosis in Korean cattle. The results obtained will be reported hereafter.

II. Statistics as to Prevalence

In order to determine whether the animals mentioned are infested with sarcosporocystis or not we examined the favourite sites of parasite, commonly the skeletal musculature viz. Musculus psoas parvus & magnus with naked eyes, often by means of microscope, about 15 to 30 heads of calves being usually examined in every week or frequently twice in a week. The results obtained are shown in the following table.

Table 1

<table>
<thead>
<tr>
<th>Years</th>
<th>Examined</th>
<th>Infected (%)</th>
<th>Non-infected (%)</th>
</tr>
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<tbody>
<tr>
<td>1924-1925</td>
<td>204</td>
<td>197 (98.6)</td>
<td>7 (3.4)</td>
</tr>
<tr>
<td>1925-1926</td>
<td>628</td>
<td>619 (98.6)</td>
<td>9 (1.4)</td>
</tr>
<tr>
<td>1926-1927</td>
<td>569</td>
<td>562 (98.8)</td>
<td>7 (1.2)</td>
</tr>
<tr>
<td>1927-1928</td>
<td>621</td>
<td>515 (88.8)</td>
<td>6 (1.2)</td>
</tr>
<tr>
<td>Total</td>
<td>1922</td>
<td>1893 (98.6)</td>
<td>29 (1.5)</td>
</tr>
</tbody>
</table>

Note: The previous examination reported in 1926 revealed 97% of infested cases out of 250 heads of Korean cattle. During those periods we could not found so distinct seasonal variation with respect to the occurrence of parasite as described by Bergmann (1913) of Shweden regarding Sarcocystis miescheriana in swine. On the other hand, the infestation was found to be slightly serious in the calves used for rinderpest experiment for a long time in clean stall, and this fact is in an agreement with the observation made by Scott (1918-1920) of America, regarding Sarcocystis tenella in sheep. 5 to 9 years old hyperimmunized oxen, anti-rinderpest serum producers, enclosed in a stall for 1-5 years always showed
heavy infestation of sarcosporidia. The writer observed slight sarcosporidian infestation in the healthy cattle slaughtered at the municipal abattoir of Fusan city, the exact feature and a comparison of them will be made for a further communication. Of a number of swine examined in our laboratory some adult ones, over one year old, were found to be infested with the same parasite as bovine sarcocystis, but young pigs showed no parasites at autopsy.

III. TOXICITY OF BOVINE SARCOSPORIDIA

The animal experiments have been done employing many adult rabbits regarding the toxicity of bovine sarcosporidia A, B and C. The material used for this experiment consisted of 1 per cent glucose and normal saline solution and the whole cyst, taken aseptically from the psoas muscle fibres, macerated and filtered through a gauze. The suspension was injected into the ear vein of the animals. It was found that one fresh cyst was sufficient to kill the rabbit within 24 hours. The animals which received the injection manifested the following symptoms, viz. uneasiness, irritation or excitement, accelerated pulse, rapid respiration, elevation of body temperature, spiritless or dullness, loss of appetite, severe diarrhoea, paralysis, paresis or weakness of the hind legs, coma, collapse which followed death. The gross pathological changes were softness of the cerebrum and cerebellum, congestion of the heart and kidneys, slight inflammation of the intestines and swelling of the liver with bloody or green bile.

The survived rabbits treated with more doses manifested slight symptoms or reactions and appeared to have achieved a little active immunization.

A calf which had received by route of the jugular vein 40 c.c. of the material, actually containing 80 cysts of C form of bovine sarcosporocystis, showed no remarkable symptoms except tympanitis which occurred periodically showing chronic gastric disorder, but marked hyperleucocytosis and hypereosinophilia. Two months later, at the post-mortem examination of the calf, no development of the parasite either in the internal organs or in the skeletal musculature was found.
IV. CLASSIFICATION OF BOVINE SARCOSPORIDIA

The taxonomic explanation of sarcosporidia is very difficult work in the present protozoology. The development or life history and the other characteristic distinction amongst the allied organisms are still in darkness. Sarcosporidia are commonly classified into many species according to their host but one host is often infested with much distinguishable two or more forms of sarcosporidia. Having studied carefully the morphology of the bovine sarcosporidia, paying much attention to their domicile and the pathological changes of the organ or tissue attacked by them, I have found that it is more convenient in the study of this parasite to classify them into 2 types and 3 different kinds A, B and C, not using the word "genus or species".

The explanation of these 3 kinds is shown in the following table.

Table II. Explanation of the bovine sarcosporidia, A, B & C

<table>
<thead>
<tr>
<th>Kinds</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence</td>
<td>very rare (0.02%)</td>
<td>most common 98.5% in calves 100% in oxen</td>
<td>frequent 2% in calves 50% in oxen</td>
</tr>
<tr>
<td>Form</td>
<td>round, oval or spherical</td>
<td>long, slender hairy or rod-like</td>
<td>long, spindly form like <em>Oxyuris vermicularis</em></td>
</tr>
<tr>
<td>Dimensions of cyst (μ)</td>
<td>200—400</td>
<td>200—260× 1500—2500</td>
<td>400—900× 4000—9000</td>
</tr>
<tr>
<td>Sickle (μ)</td>
<td>2—3× 7—8</td>
<td>4.5—6× 5—14</td>
<td>4.7—7.0× 13.3—27.5</td>
</tr>
<tr>
<td>Tissue reaction</td>
<td>marked cell infiltration</td>
<td>slight or no cell infiltration</td>
<td>slight or no cell infiltration</td>
</tr>
<tr>
<td>Toxicity to rabbit</td>
<td>?</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>Cyst membrane</td>
<td>nonstriated fibrous, hyalin lining</td>
<td>striated outer, hyalin inner</td>
<td>striated outer, hyalin inner</td>
</tr>
<tr>
<td>Sites attacked</td>
<td>connective tissue, scrous &amp; mucous membrane, organs, vascula</td>
<td>heart, skeletal musculatures</td>
<td>heart, skeletal musculatures</td>
</tr>
<tr>
<td>Affinity</td>
<td><em>S. besnoiti</em> (<em>besnoiti, globidium besnoiti</em>) (Besnoit &amp; Robin, Franco &amp; Borges) in cattle; <em>Fibrocystis tarandi</em> (Hadwen) in reindeer; *Sarcocystis var. (Bennett) in horse.</td>
<td><em>S. miescheriana</em> (Kühn) in swine; <em>S. tenella</em> (Railliet) in sheep; <em>S. muris</em> (Blanchard) in mouse; <em>S. hirsuta</em> (Moule) in cattle, etc.</td>
<td><em>S. blanchardi</em> (Ogden) in buffalo; <em>Balbiania gigantea</em> (Reillard) <em>S. tenella</em> (Bergmann) in sheep, etc.</td>
</tr>
</tbody>
</table>

*Note*: The form A occurred only in one case of calf.
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V. MORPHOLOGY OF THE BOVINE SARCOSPORIDIA

The morphological study of sarcosporidia belongs to one of the most difficult things, but I tried to examine the structure of the parasite by means of a simple technique which is so practical as any laboratory men can do. Our investigation was carried out as follows: The parasite was taken out from the psoas muscle, etc. of the animal immediately after slaughtering, then prepared, aseptically by a fine needle under naked eyes or microscope in an isotonic neutral solution. Slide preparations were made for observing the morphology in the fresh state as in the muscle fibres, and on the other hand, the parasites were treated with chemicals or fluids and preserved at varied temperature, viz. in room, ice box (about +5°C), and incubator (30° to 38°C). As fluid we used saline (0.4—1%), glucose (1—5%), Roger's solution, Ringer or Lock solution, cattle serum, saliva, bile, digestive juice, etc. As chemicals, tincture of iodine, potassium iodide, Gram solution, glycerin, acids or alkalies, etc. were employed. The preparate was stained after Romanowsky, Leishman, or with Giemsa, Gram and other anilin dyes etc.; hanging drops or sometimes unfixed coloured preparations were also observed.

By carrying out these procedures I found out many interesting changes as regards the morphology of the parasite. One of these polymorphous changes of parasite may belong to the auto-regulating or adaptation phenomenon or the abnormal cycle of development of the complete or unripened premorphous living cells. The appearance of another change may be attributed to the presence of direct products or by-products of the physico-chemical reaction of the organic substances of parasite treated.

VI. CYST WALL

I may point out here that the outer membranes of the cyst have more or less resistance to treatment while the inner content including the so-called spore or sickle bodies is very sensible to slight stimulation. There may be remarked in the cyst wall two layers of membranes, the hyaloid-amorphous inner membrane and the fine cyst chamber wall. These may be of same nature in
their origin and may necessarily be produced by the parasite itself completing their schizogonical development in the intra-muscular fibres of animals. The outer coat which may partly be originated to the host tissue is in most cases undergoing varied degree of degeneration and takes only eosin dye.

The fine, swollen, hyaline, transparent and striated outer coat of sarcocyst can easily be decomposed by chemicals, and sometimes may be separated by acetic acid (1%) from the inner membrane leaving there a free unbroken chamber. It may be said, that the outer coat is a newly degenerated muscle prefibre in contact with the normal prefibres (myofibrils). Along the coat there may be remarkable an abundant rest of prefibre membranes (myofibrila epimembranes) covering the inner membrane of cyst. When the destruction occurs in the inner membrane or chamber wall the contents will flood out. The thickness of the inner membrane and chamber wall will mean the age of cyst. Thus, one may often notice the presence of middle layer in the old sarcosporidian cyst. On account of this construction of cyst wall membranes, if a further spread of the parasite may occur in the musculature of the same individual, there must be degeneration or rupture of muscle fibres causing destruction of cyst. It may be an interesting fact from the pathological point of view that in cases of anaemia, some chronic disease, parasitic disease, nutrient deficiency, “scrapie” in Scotland, “lamziekt” in South Africa, etc. which always accompany more or less pronounced muscle degeneration sarcosporidiosis is proved to be prominent.

VII. CYTOLOGY AND BIOLOGY OF SICKLE

The young Miescher’s sacks, 16—26 μ or sometimes 10—15 μ in width, were often found microscopically. Generally the content varies with the dimension of cyst. In very fresh state they were always found as round or spherical uninucleated cytoplasmic bodies measuring 4—4.5, 5.4—6.2 and 6.5—8.5 μ in diameter. These lymphocyte-like globules disclosed later very complicated morphological changes. Usually their granules and nuclei became soon distinct, and their thin transparent capsules with their ectoplasm showed much permeability and less elasticity.
condition of temperature and media they proliferated by simple division or sometimes by budding or chain formation, then each of those proliferated bodies changed by peculiar process into the sickle form which was mononuclear, metamorphogranular and furnished with blunt and tapering ends. In some cases vacuoles were seen in the sickle. By employing the technique of McGowan (1923) (1% glucose solution), it was found that the division of nucleus and endoplasm was more marked as compared with that of the ectoplasm or the capsular membranes, so there were demonstrated besides the sickle forms and many broken particles, oval, elliptical or round and binucleated blast forms. These spherical bodies appeared to have two layers of thin transparent membranes which were clearly demonstrated by tincture of iodine or carbolized anilin dyes but they were quite different from those of the spore of microsporidia, coccidia or blastomyces.

I may say that sarcosporidia living in the muscle fibres proliferate there by the mode of “schizogony” and the uninucleated plasmodial globules may be “schizont” which may develop into a few “meronts” in an abnormal condition. The so-called “sickle” with one nucleus may be considered as a “merozoit”, not a spore, and the encysted binucleated round forms as “meroblasts”. The “gametocytes” in the leucocytogregarina of wild rat, reported by S. Kusama, K. Kasai & R. Kobayashi (1919) could not be demonstrated.

The morphological and cytological observation of sickle bodies made by many authors gives almost same result as in my investigation regarding nucleus or granules, etc. I should like to write here some new findings which must be added to the mysterious nature of sarcosporidian sickle. The sickle was found to be more or less Gram positive, and to have many granules easily stained by iodine especially scattered at its middle part. In the prepare of fresh materials treated with tincture of iodine I recognized almost in each sickle one or two spore-like bodies which are small, round or ellipsoidal, aplasmic and bright. They were found to be stained very hard and to have more or less resistance. All of them were noticed to be surrounded by dark brownish yellow mass of cytoplasm and to be furnished with peculiar long filament,
so that these bodies appeared to be polar capsules of microsporidia or cnidosporidia. These bodies were 0.78—1.0, 1.4—1.8 or 0.9—1.1 \( \mu \) long. The length of filament measured 60—90 \( \mu \) and their width 0.3—0.4 \( \mu \). It must be pointed out that these filaments were rarely found but the spore–like bodies or polar capsules were not of rare cases.

It will be worthy of note that an active movement took place when the sickles were mounted with the bile of guinea–pig. Fresh cysts taken from a heavy infested calf muscle were kept in the following fluid, viz. 1 part of inactivated calf serum mixed with 19 parts of 0.4\% saline solution at 25—27\( ^\circ \)C for an hour. Then they were treated with fresh guinea–pig bile and observed at room temperature, or under warmed microscope or in dark field apparatus. There were remarked two sorts of movements; the one was a circular or pushing movement, the sharp end of sickle being ahead and the whole body being more or less elongated in its curved position. One or four motions were needed to finish one circulation but sometimes one motion caused two or three circulations. The other kind of movement was noticed to be very active, the sickle quickly pushing forwards, or spirally swimming as if the halibut does in the water, and not as the movement of trypanosoma. These active movement continued for two to three hours under microscope in summer, viz. at about 26\( ^\circ \)C, but after 18 hours they were found to be less active in only a few numbers of sickles preserved in the same fluid. The movement above mentioned was also observed by S. Sato (1921) in \textit{S. blanchardi} treated with pancreatic juice, but in my case the movement was found to be more active. The osmic acid preparation showed, however, neither flagella nor any motile organ.

Infection experiments, culturing and blood and serum examination have been carried out in connection with those mentioned above but they will be made for a future communication.

\textbf{VIII. Summary}

1. I have studied the frequency of occurrence of bovine sarcosporidia in native Korean cattle, and the toxicity of the cyst in fresh condition, and classified the parasite into two types and
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three kinds.

2. Some new facts were observed regarding the structures of cyst wall and cytology and biology of sickles.

Our thanks are due to Dr. T. Mochizuki, Director for his encouragement, and Dr. C. Kakizaki for his special help, and Messrs. G. Mizuki, M. Sasaki, Nishimura, Hirano, S. Namikawa, K. Naruse, J. Nakamura and all members of our institute for their aids and especially Y. Toshijima for his assistance in this work.

EXPLANATION OF PLATE

Fig. 1. Transverse section of skeletal musculature, embedded in celloidin, stained with hematoxylin-eosin.

Fig. 2. Bovine sarcosporidia of slender kind separated from muscle fibres, in fresh condition. Low magnifying power

a: Bovine sarcosporidia of spherical form (A).
b: Bovine sarcosporidia of long type and slender kind (B).

inf.: Cell infiltration.

Fig. 3. Bovine sarcosporidia of long type and slender kind, a fresh slide preparation mounted in Japanese black ink (1/12 × 2 oil immersion). Note the minute structure of outer membranous substances, over-flooding of contents in one portion and the black points where the striated outer coat had been lost.

Fig. 4. Film preparation of bovine sarcosporidia, stained by acid Giemsa (× 1300). Note the leishmania form of spores.

朝鮮牛ノ住肉胞子虫ニ關スル研究

中 西 俊 藏

（朝鮮總督府獸疫血清製造所 所長 農學博士 望月楨三）

（昭和4年5月10日受付）

朝鮮牛ノ筋肉＝住肉胞子虫ノ多數寄生スルコトハ曩キ＝著者ノ認メタル所ナリ今更＝此ノ寄生體＝就キ下記ノ如キ成績ヲ得タリ

1. 1924年ヨリ1928年＝至ル滿4年間當所＝於テ年齢10—20
ケ月前後ノ朝鮮産兎ノ住肉胞子虫寄生＝関スル検査ヲ行ヒ寄生率98.5％ナルヲ発見ス期節ト寄生率トノ関係显著ナルス＝雖モ牛疫関係試験＝ラ長ク舎内＝繁殖セラレタル動物ノ感染程度ハ更＝著シキモノアリ

2. 多数ノ動物試験ノ結果ヨリ1個ノ新鮮採取牛ノ住肉胞子虫胞囊ハ成兎ヲシテ24時間内＝中毒瀕死セラムル毒性ヲ有スルコトヲ確メタリ

3. 牛ノ筋肉＝於ケル住肉胞子虫ヲ2形態3種類(A, B, C)＝區別シ得タリ

4. 寄生體ノ形態及び細胞構造ヲ追求スル方法トシテ常＝新鮮ナル材料ヲ用ヒ種々ノ條件置ノ下＝顕微鏡的検査ヲ行ヒ被膜ノ構造及び内容ノ性質＝關スルニ三ノ知見ヲ得タリ

5. 胞囊被膜ハ＝本質上内外ノ2層＝區別セラレル内膜層＝寄生體自身＝必要構成膜質＝シテ外膜層＝寄生體ノ＝＝変性セラレタル筋纖維ノ一部ヲ本末＝宿主ノ組織ヨリ誘導セラレタルモノト認メル

6. 胞囊内容ノ形態及び生物學的研究＝ヨリ牛ノ住肉胞子虫ノ筋纖維内＝於ケル正規ノ増殖機轉ハ無性分裂増殖＝ヨルモノ、如ク極メテ新鮮ナルモノハ内容何レモ球状單核細胞性無性分裂仔ヨリ成ツル外的感作ハ＝レリ1〜2個ノ分芽仔＝移行シ1核特異細胞所謂鱗狀體ヲ形成シ又ハ2核ノ分芽胚ノ形態ヲ呈スル＝至ラシム

7. 鰤状體ハ＝たらむ胞芽性、液度染色顕粒ヲ有ス液度＝弾質＝＝ヨリ1個又ハ2個ノ小芽胞様體ヲ形成シ稀レ＝長キ索絡弾状形成物ヲ生ズ又活潑ナル運動ヲ発現スル事實ヲ発見セリ

（自抄）