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Sarcocystosis in a 30 month old Hanwoo(*Bos taurus oreanae*)

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Abstract

Unusual yellowish-green intramuscular granulomatous lesions were found in a carcass of Hanwoo (slaughtered, 30 month old). Those were 1-3 mm in diameter, oval shaped, and paralleled with muscle fibers. Histologically, severe inflammation, eosinophilic granulomas and necrosis were observed in the muscle tissue. We also observed sarcocysts in the muscle cells. In a polymerase chain reaction, we identified 900bp length, *sarcocystis* specific fragment. It would be diagnosed as sarcocystosis in Hanwoo.

Key words : Cattle, *Sarcocystis*, Sarcocystosis, Eosinophilic myositis

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Introduction

Sarcocystosis is a worldwide parasitic disease that is caused by *Sarcocystis* [1,2]. It has two life cycles, intermediate and definitive host. It proliferates asexually in the intermediate host and proliferates sexually in the definitive host. It has a wide host range but host specificity by species is very high. For example, intermediate host of *Sarcocystis cruzi* is cattle and definitive host is canine species. Intermediate host of *S suihominis* is pig and definitive host is primates. And they don't infect other species. Infected definitive host excretes sporocyst in feces and intermediate hosts are infected when they eat polluted pasture or water with those feces [1]. Merozoites of *sarcocystis* proliferates in muscle or nerve tissues of intermediate hosts and they form intracellular sarcocyst. Many bradyzoites, infectious form of *sarcocystis*, exist inside the sarcocyst [1]. Definitive hosts are reinfected when they eat raw meat of infected intermediate host. *Sarcocystis* can cause gastrointestinal disturbance in definitive hosts but usually inapparent. Infected intermediate hosts can experience muscular pain, fever, anemia, cardiomyopathy, muscle tremor, recum-bent etc., but mostly inapparent, too [1,2].

Even though it doesn't induce severe clinical signs, it is a important disease in

a public health aspect because some *sarcocystis* species can infect human. And, it is important economically as well because most carcasses which are detected upon inspection are condemned.

Detecting merozoites or sarcocyst through histological study are good diagnostic methods [1]. But ultrastructural study under the electro-microscope or gene sequencing should be performed for species identification [3-5].

Examination

June 2008, a farmer sent 16 heads of 30 month old Hanwoo to the slaughterhouse in Gyeonggi province. And yellowish green granules were found in one carcass among them. Granules were 1~3 mm in diameter and oval shaped. They were arranged parallel with muscle fibers (Fig 1). Surrounding muscles were seen normal with the naked eye.

Suppurative bacterial infection, fungal infec-tion or parasite infection were suspected as causes that can induce similar lesions. We collected muscle samples and smeared on several types of media for bacterial and fungal study. Those were fixed on 10% neu-tralized formaldehyde solution for histological study and were stored at -20℃ for molecular biological study. For bacteriological study, we smeared granuloma at Blood agar plate (BAP), MacConkey agar(MCA) and Nutrient broth (NB) with sterilized instruments and incubated them in a 37℃ aerobic incubator. After a day, we subcultured NB to MCA and BAP, and incubated them in the same condition. All media were observed twice a day for 3 days. For fungal study, we smeared same lesion at Sabouraud dextrose agar (SDA) in same method. Incubation condition was aerobic, room temperature and humid. We incubated it for 7 days and watched it twice a day. For histological study, we embedded formalin fixed samples in paraffin blocks and cut them 3㎛ in thickness and stained with hematoxyline and eosin (HE). Each slides were read with light microscope (Leica DM2500) and some slides were photo-graphed with Leica DFC420C.

Non of the microorganisms grew in the media. Severe inflammation, especially eosinophilic myositis, was found in histological study (Fig 2). There were severe inflammatory cells infiltration and remnants of necrotized muscle fibers in the center of granulomas. In one granuloma, a degenerative parasite cyst was found. Outside of granuloma was surrounded by fibrous tissue and epithelioid cells. Lots of inflammatory cells were infiltrated outside of granuloma, too. They were infiltrated along the muscle membrane.

Inflammatory cell infiltration was more severe as they get closer to granulomas. Most muscle tissues were atropic and edematous and muscle cells near granuloma were degenerative and necrotic. Some necrotic muscle fibers were phagocyted by macro-phages and emptied spaces were replaced to connective tissue (Fig 2). Seven intramuscular parasite cysts were found among 24 slides. We supposed it as sarcocysts based on location, shape and size of the cyst [1,5]. Sarcocysts were located inside of muscle fibers. They were long oval shape and paralleled with muscle fibers. Their shapes

Fig 1. Lots of small yellowish green granulomatous lesions are spreaded in the muscle.

were almost complete circles where they were cut transversely and diameters were 55~94.9㎛. Sarcocyst walls were thick (6.6~ 8.1 ㎛) and consisted of thin inner membrane and thick outer villa protrusions. Center of sarcocysts were filled with lots of bradyzoites, infectious form of *Sarcocystis*. Bradyzoites were half moon shaped, stained basophilic. They were comparted with eosinophilic septa. Some metrocytes were observed near a

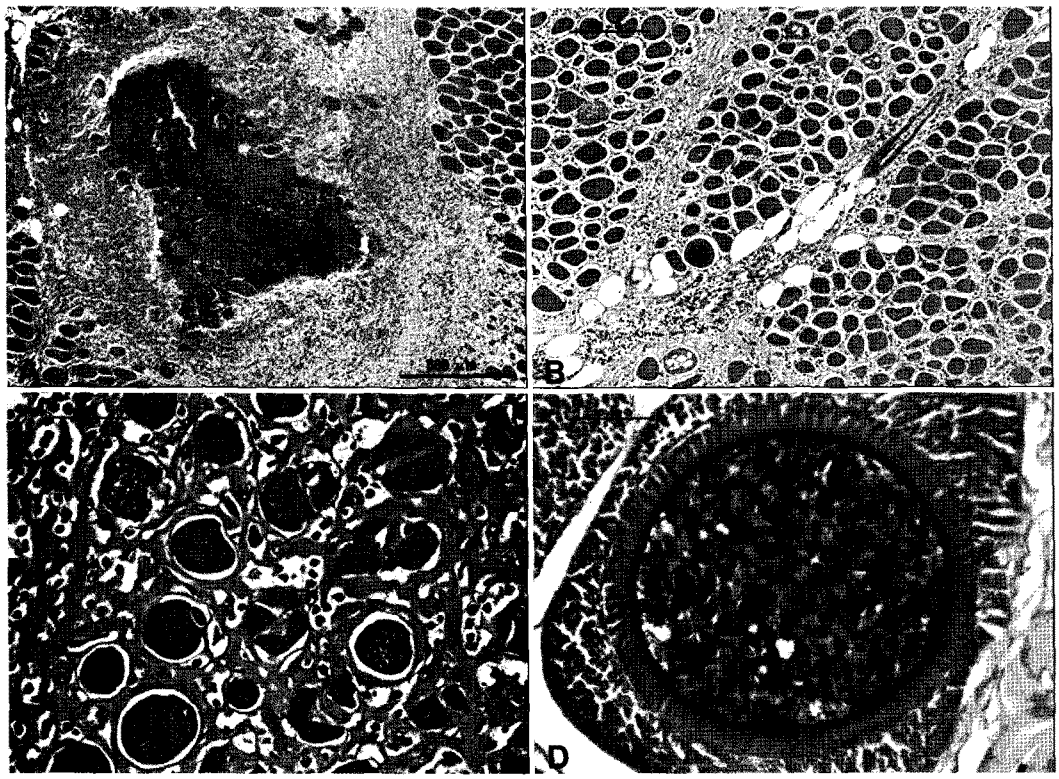

Fig 2. Eosinophilic granuloma in muscle. Degenerative eosinophils, necrotic muscle fibers are aggregated in the center and epithelioid cells and fibrous tissue are surrounding them. Inflammatory cells infiltrated in peripheral tissue, too. Muscle fibers outward granuloma are degenerated(A: HE. ×50). Degenerated muscle tissue. Infammatory cells are infiltrated along the muscle membrane. Muscle cells are degenerated and somes are necrotized. One sarcocyst is observed inside of a muscle cell(B: HE. ×100). Muscle necrosis and replacing to connective tissue. Macrophages are phagocyting necrotized muscle cells and emptied spaces are replaced to connective tissue(C: HE. ×400). Cross section of Sarcocyst in a muscle fiber. Lots of bradyzoites are aggregated in the center. They are comparted by thin eosinophilic septa. Sarcocyst has thick wall. It is composed of thin inner membrane and thick outer villa protrusion(D: HE. ×1000).

basement membrane. Metrocytes were basophilic but it was stained weaker than bradyzoites (Fig 2). There are three *Sarcocystis* species that can infect cows as intermediate host[6]. They are *Sarcocystis cruzi, S hominis* and *S hirsuta*. Among them *S hominis* and *S hirsuta* form thick wall sarcocyst. It needs electromicroscopical analysis or gene sequencing to confirm the correct species [3-5,7].

We performed s*arcocystis* specific PCR. We collected sarcocysts from muscle samples and extracted DNA with DNA extrac-tion kit (AccuPrep Genomic DNA extraction kit; Bioneer, Korea). Extraction method was followed by manufacturer's instructions. A set of primers, 18S2L (forward primer : 5' GGA TAA ACC GTG GTA ATT CTA TG 3') and 18S3H (reverse primer : 5' GGC AAA TGC TTT CGC AGT AG 3'), were used for amplify a 900bp long part of 18s rRNA gene of *Sarcocystis*[7,8]. For PCR reaction, we mixed 4㎕ of the DNA extract, 30pM of each primers (100pM/㎕ solution), PCR premix (AccuPower HotStart PCR Premix; Bioneer, Korea) and 15.4㎕ of RNase free water. Total volume was 20㎕. The PCR was performed as follows (PTC-200, MJ research) : 94℃ for 5 min, followed by 40 cycles of 94℃ for 40sec, 52℃ for 30 sec, 72℃ for 60 sec, followed by 72℃ for 5 min. The PCR product was analysed by using agarose gel electro-phoresis.

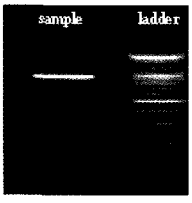

Fig 3. The result of *Sarcocysis* specific PCR 900bp, *Sarcocystis* specific fragment was observed (left : *Sarcocystis* specific fragment from muscle sample, right : 100bp DNA ladder(bioneer, Korea)

As a result of the PCR, we observed 900 bp length, *sarcocystis* specific fragment (Fig 3).

Discussion

A farmer of this case feeds about 50 steers on his farm, he raises only castrated Hanwoo. He buys 6 month old calves from other farms and feeds them for 24 months before he sends them to the slaughterhouse. He didn't raise any dogs or cats for several years and didn't pasture cattle in pastureland. These epidemiological situations strongly suggest that s*arcocystis* infection might happen before the calf came to his farm.

Sarcocystis infection rate in Africa and Southeast asia is very high. In some areas of them, it reaches to 80~100% [1,2]. In case of Korea, the rates were 41.5% and 36.7% in 1988 and 1990, respectively [6,9]. But, there wasn't any reported case after those times. So, some researchers think s*arcocystis* doesn't exist in Korea now. In fact, that thought is somewhat reasonable because most Korean farmers prefer to raise their cows in the pen rather than pastureland and indoor raising could block the chance of *sarcocystis* infection effectively. But, *sarcocystis* infection rate in Korea maybe higher than they expect because *sarcocystis* infection rate was almost 40% in the past and there hasn't any special sanitation program for decreasing *sarcocystosis*. And it is impossible to find microscopic sarcocyst unless microscopic studying is performed. It means microscopic sarcocyst couldn't be found with just simple sight inspection.

Some s*arcocystis* has human bovine life cycle. It means we can be infected to *sarcocystis* anytime when we eat row meat of infected cattle. Korean eat raw meat culturally. So we shouldn't overlook the possibility of human infection. Researches for *sarcocystis* infection rate of domestic animals and strengthening of slaughter-house inspection system are urgent.

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