

Studied on *Sarcocystis* detected from Japanese sika deer (*Cervus nippon centralis*)
in Hyogo Prefecture

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Protozoa of the genus *Sarcocystis* belong to the phylum Apicomplexa, class Sporozoa, order Eucoccidiorida, and the family Sarcocystidae. They form sarcocysts, which are large cysts that can be seen with the naked eye, in the muscles of the intermediate host. Protozoa of this genus require two hosts to complete their life cycle: a definitive host and an intermediate host. The definitive hosts include humans and other primates and carnivores, and the intermediate hosts include herbivores and omnivorous animals, rabbits, rodents, and birds. Both intermediate and definitive hosts are considered to have relatively high host specificity. Infection from the intermediate host to the definitive host is caused by consuming sarcocysts and infection from the definitive host to the intermediate host by consuming sporocysts excreted in the definitive host's feces. In Japan, animal damage by Japanese deer and wild boar in mountain-based regions is getting close attention, and they are caught by hunting and harmful control. In Japan, where the popularization of meat and livestock was inferior to that of the West, these wild animal meat has been supplied as a vital protein source since ancient times, but Japanese sika deer were caught only a little until more than ten years ago and were not as common as wild boar as food. However, the population of the sika deer has increased dramatically nationwide over the past ten years, and hunters and their surrounding people have consumed them; and recently, the effective use of the meat of Japanese sika deer has been proposed by the private and administrative authorities. However, the report on the present state of *Sarcocystis* in Japanese sika deer is not sufficient yet. This time, the opportunity of hunting of Japanese sika deer in Hyogo Prefecture was obtained to investigate the actual condition.

1. Parasitic status of *Sarcocystis* in Japanese deer (*Cervus nippon centralis*) in Hyogo Prefecture, Japan

From the 64 Japanese deer (*Cervus nippon centralis*) caught in the central mountainous area of Shimomikata district, Fukuchi, Shiso City, Hyogo Prefecture for hunting and harmful control, materials were collected by age (under 1 year old to 5 years old) and by site (myocardium, diaphragm muscle, biceps femoris, and longissimus dorsi) to investigate the parasitism rate of sarcocysts observed there. The parasitism rate in the investigated Japanese deer was as high as 81.3%. By age, there was no clear difference in the infection rate of individuals after 1 year of age, but no parasitism was observed in the young deer under 1 year of age. By site, although there were almost no significant differences in infection rates among myocardium, diaphragm muscle, hindlimb biceps femoris, and longissimus dorsi, a significantly higher parasitic density was observed in the myocardium. This result did not reveal whether the investigated Japanese deer had multiple parasitic species or a single species with certain degree of cardiac preference. The obtained sarcocysts in any parasitic site were oval in shape and varied in size (445.5-1064.3 × 99.0-247.5 μm; average of 678.6 × 174.2 μm). *Sarcocystis* observed in this investigation was morphologically different from the species previously reported in Japan.

2. Morphological study on *Sarcocystis* from Japanese deer in Hyogo Prefecture

When we examined sarcocysts in muscle to investigate the parasitism status of *Sarcocystis* species of Japanese deer in Hyogo Prefecture, differences in their size and morphology were observed. Thus, we examined the morphological features of sarcocysts using light and electron microscopes to investigate their morphology. As a result of examining the relationship between sarcocysts and the total number of bradyzoites, there was a positive correlation between the size of sarcocysts and the number of bradyzoites, suggesting that the difference in the size of sarcocysts

depends on the degree of development. The comparison of the wall structure of the cysts was considered to be useful in the morphological study of sarcocysts, and the comparison of the morphological differences of sarcocysts between different species was made on the basis of the useful comparison of the morphology of the hairy appearance seen on the outside of the cysts wall. Among the deer-derived species reported in various parts of the world, the sarcocysts detected at this time were the most similar to *S. grueneri* described from reindeer (*Rangifer tarandus*) and red deer (*Cervus elaphus*), which suggested that they might be undescribed species different from any species examined.

3. Molecular taxonomic study of *Sarcocystis* originated from Japanese deer in Hyogo Prefecture

Sarcocystis parasitizing Japanese deer in Hyogo Prefecture were divided into three types: needle-shaped, cayenne, and oval based on morphological study in Chapter 2. However, these three types were all found to be the same species by 18 *S rDNA* genetic analysis. The taxonomic position of this *Sarcocystis* was studied using 18 *S rDNA* and *cox1* genes. As a result of the classification of *Sarcocystis* protozoa originated from deer by the *cox1* gene, this *Sarcocystis* formed a clade independent of those previously reported. This indicated the possibility of this species being the single species which has not been reported until now from the genetic analysis.

4. Experimental infection of dogs with *Sarcocystis* from Japanese deer in Hyogo Prefecture

We had an opportunity of performing an experimental infection by giving myocardium of Japanese deer containing sarcocysts detected from the muscle of

Japanese deer in Hyogo Prefecture to two dogs. The purpose is to confirm whether infection is established and the number of days in the prepatent period. Excretion of sporocysts, which were not detected before the administration of sarcocysts, was confirmed on days 5 and 6 after administration. This proved that the dog becomes the definitive host, and the prepatent period of this species is 5-6 days. These results suggested that *Sarcocystis* detected in Hyogo Prefecture is a new species different from the known species based on morphological, genetic, and prepatent period differences, and revealed that the life cycle of this species is maintained by canids as the definitive host and deer as the intermediate host.

The results of this study showed that *Sarcocystis* in the Japanese deer in Hyogo Prefecture had an infection rate of more than 80%, and the Japanese deer were found to be intensively infected. It was also found that the species different from those detected from other regions in Japan mainly inhabit. The life cycle revealed that canids are the definitive host and the deer are the intermediate host, indicating the necessity of paying sufficient attention to the role of hunting dogs in hunting and the use of deer meat as edible meat from game.