Preparation and Applicability of *Sarcocystis cruzi* Antigens and Their Anti-*S. cruzi* Rabbit Sera for Serodiagnosis of Bovine Sarcocystosis

Morihiro SAITO, Yoshinao OHUCHI¹⁾, Masaru KOBAYASHI²⁾, Makoto HARITANI³⁾, and Hiroshi ITAGAKI⁴⁾
Kumagaya Meat Inspection Office Saitama Prefecture, Shimomasuda, Kumagaya, Saitama 360, ¹⁾Northern Animal Health Center, Nakagachi-cho, Mito, Ibaraki 310, ²⁾National Institute of Animal Health, Kannondai, Tsukuba, Ibaraki 305, ³⁾Tohoku Branch Laboratory, National Institute of Animal Health, Shichinohe, Kamikita, Aomori 039-25, and ⁴⁾Department of Parasitology, Azabu University School of Veterinary Medicine, Fuchinobe, Sagami-hara, Kanagawa 229, Japan
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ABSTRACT. *Sarcocystis cruzi* antigens and their antisera were prepared for the seroimmunological diagnosis of bovine sarcocystosis. Fresh *S. cruzi* cysts directly removed from cardiac muscle of slaughtered cattle were digested with trypsin to release bradyzoites. By twelve cycles of freezing at -22°C and thawing at 37°C, bradyzoites were let to leach out soluble material. The soluble antigens were inoculated four times to rabbits at a dose of 343 µg protein and anti-*S. cruzi* sera were prepared with blood of the rabbits. Gel immunodiffusion test showed no cross-reaction between the present antigens and any of anti-*Toxoplasma gondii*, -*Hammondia hammondi* and -*Besnoitia wallacei* rabbit sera. Avidin-biotin complex immunoperoxidase (ABC) technique with the present anti-*S. cruzi* rabbit sera showed clear positive reaction against *S. cruzi* cysts in the muscular sections of infected cattle.—**KEY WORDS:** anti-*Sarcocystis cruzi* rabbit sera, preparation, *S. cruzi* antigen.

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Chronic *Sarcocystis* infection has been immunologically confirmed in slaughter cattle in Japan [5]. In meat inspection *Sarcocystis* infections have been diagnosed by microscopical examination of sarcocysts and/or bradyzoites or immunological techniques such as avidin-biotin complex immunoperoxidase (ABC) technique, whereas in field survey cattle have been examined for the infection by immunological techniques such as gel-diffusion and indirect agglutination tests [3]. These techniques, however, are not sufficient for accurate diagnosis of the infection and therefore, more sensitive tests such as ELISA should be applied.

We attempted to prepare more purified antigens of *S. cruzi* and their antisera for more accurate seroimmunological diagnosis of *Sarcocystis* infection in cattle.

Preparation of *S. cruzi* antigens: A total of 7,500 fresh *S. cruzi* cysts were directly removed from cardiac muscle of slaughtered cattle under a microscope [4]. The fresh cysts were digested in 0.2% trypsin PBS solution at 37°C for 20 min and the solution was centrifuged at 3,000 rpm for 10 min. The pellet was suspended in an adequate volume of PBS and the suspension was centrifuged at 3,000 rpm for 10 min. After two cycles of centrifugation, the resultant pellet was mixed with the same volume of PBS, and the mixture was frozen at -22°C and then let to thaw at 37°C. By repeating freezing and thawing twelve times, soluble material was leached out.

The total protein of 7,500 sarcocysts weighed 3,250 µg by Lowry method.

Preparation of anti-*Sarcocystis cruzi* rabbit sera: *S. cruzi* antigens were subcutaneously injected to rabbits four times at weekly intervals in a dose of 343 µg protein mixed with five times volume of complete Freund's adjuvant. Two weeks after the last injection of the antigens all blood was obtained from the rabbits after antibodies were preliminarily titrated by a modification of Ouchterlony test.

Specificity of the antigens: Gel immunodiffusion test with the present antigens showed no cross reaction against

any of anti-*Toxoplasma gondii*, -*Hammondia hammondi*, and -*Besnoitia wallacei* rabbit sera but it showed positive reaction against the present anti-*S. cruzi* rabbit sera at eight fold dilution (Figs. 1, 2).

Detection of intramuscular sarcocysts by ABC technique with anti-*S. cruzi* rabbit sera: Cardiac muscle blocks of slaughtered cattle infected with *S. cruzi* were fixed in 10% formalin, embedded in paraffin and sectioned. The sections were examined by ABC technique [2] with an Elite ABC Kit (Vestastain). Sarcocysts in the paraffin sections exhibited intense reaction when anti-*S. cruzi* rabbit sera diluted 1:2,048 was used as the primary sera (Figs. 3, 4).

Bradyzoites for *Sarcocystis* antigens have been har-

Fig. 1. Measurement of anti-*S. cruzi* rabbit sera titers by gel diffusion precipitin test. Sera in the peripheral well are those against *S. cruzi* at different dilutions expressed in figures. The central well contains *S. cruzi* antigens.

Fig. 2. Cross-reactions by gel diffusion precipitin test. Antisera in the peripheral wells are those against each coccidian species at different dilutions shown in figures. The central wells contain *S. cruzi* antigens.

Fig. 3. Intense positive reactions at *S. cruzi* cysts to anti-*S. cruzi* rabbit sera by avidin-biotin immunoperoxidase complex (ABC) method ($\times 200$).

Fig. 4. Reactions at *S. cruzi* cysts by ABC method without anti-*S. cruzi* rabbit sera as the primary sera ($\times 200$).

vested by digestion of infected meat with pepsin and centrifugation of Percoll-zoite suspension to clean bradyzoites of host tissue [1, 3]. This method is complicated in process and the material used is infected meat, so the antigens might be contaminated with the protein originating from host tissue. In the present study, on the other hand, contamination of the present antigens with host tissue could be lowered as little as possible and also the procedure could be simplified because the antigens were made from fresh bradyzoites directly removed from infected muscle.

The present results show that *S. cruzi* can be immunologically differentiated from other related coccidian species with the present antigens, and that more sensitive differential diagnosis of *S. cruzi* infection can be attained by ABC technique and serodiagnosis such as gel diffusion precipitin tests and ELISA with the present antisera in meat inspection and field survey, respectively.

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