Production and characterisation of monoclonal antibodies for species diagnosis of sarcosporidial* 

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Summary: Monoclonal antibodies were raised against cystozoites of Sarcocystis muris and characterised. Twelve monoclonal antibodies reacted in the ELISA, Dot-ELISA and IFAT only with homologous antigen. The other twelve showed cross reactions of various degrees with cystozoites of S. gigantea, S. tenella, S. arieticanis, S. capracanis, S. miescheriana and S. suihominis. Proteins of S. arieticanis, S. tenella, S. gigantea, S. capracanis, S. muris and Toxoplasma gondii were compared by isoelectric focusing and showed pronounced differences.

KEYWORDS: Dot-ELISA - ELISA - Immunoelectron microscopy - Indirect immunofluorescence - Sarcocystis.

Sarcosporidia are coccidia with an obligatory two-host life cycle. Asexual multiplication takes place in the herbivorous and omnivorous intermediate hosts, resulting in the formation of sarcocysts (Miescher's tubules) in muscle tissue. Cystozoites liberated from muscle tissue undergo sexual multiplication in the carnivorous final hosts, resulting in the development of sporulated oocysts. At present 122 species of Sarcocystis have been named, but the intermediate and final hosts are known for only 56 species (5).

Sarcosporidia are widespread among farm animals. Cattle, sheep, goats, pigs and horses may each be invaded by 2-4 Sarcocystis species differing in pathogenicity. Some species cannot be distinguished by common microscopic or serological techniques. The definitive hosts are dogs, cats and humans. The pathogenic species, which are mainly transmitted by dogs, but also by humans, produce a dose-related acute disease, which may often be fatal in non-immune animals. Pregnant animals may abort. Chronic infections have an adverse effect on growth, metabolism and meat quality (1, 2, 3). Because of the inadequacy of diagnostic procedures, there are no reliable data on the economic losses caused by sarcosporidia. In the USA it has been estimated that S. cruzi alone causes an annual loss of 100 million dollars in cattle (4).

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The purpose of our research project is to investigate the species-specific diagnosis of sarcosporidia by using monoclonal antibodies (MAB's) as follows:

- by developing a simple procedure for post-mortem differentiation of morphologically identical cystozoites and other stages of sarcosporidia;
- by developing a serological test for species-specific diagnosis *intra vitam*.

Another distant objective is to use MAB's to develop a simple test for circulation antigen, and consequently acute sarcocystosis *intra vitam*, and a diagnostic test applicable to the placenta or the fetus in cases of sarcosporidial abortion.

We have obtained adequate supplies of *Sarcocystis muris* (mouse-cat cycle), *S. arieticanis* (sheep-dog), *S. tenella* (sheep-dog), *S. gigantea* (sheep-cat), *S. capracanis* (goat-dog), *S. hircicanis* (goat-dog), *S. suihominis* (pig-man) and *S. miescheriana* (pig-dog), preserved in liquid nitrogen. The starting material for all species is limited to cystozoites. It is planned to obtain material from sporozoites by excystation and of schizonts by cultivating these stages *in vitro*.

To start with, research has concentrated on *S. muris*, because it is easy to keep infected mice in the laboratory, and this species can serve as model for other *Sarcocystis* spp.

MAB's have been prepared against *S. muris* and *S. arieticanis*. Thus far, only antibodies to *S. muris* have been characterised in detail, in collaboration with the Institute of Medical and Veterinary Science in Adelaide, South Australia (Dr P.J. O'Donoghue, R. Lumb and P. Smith). Three MAB's against *S. muris*, belonging to immunoglobulin subclass IgG1j, have recognised homologous antigen epitopes of apparent molecular classes 17,000, 31,000 and 35,000 in western blots. They did not cross-react with antigens from *S. gigantea*, *S. tenella* or *S. miescheriana*. Immunoelectron microscopy showed that the antibodies reacted only with antigens of the pellicle or micronemes of the homologous species.

In addition, 24 MAB's to *S. muris* have been examined for species specificity using an enzyme immunosorbent assay (ELISA), Dot-ELISA and an indirect immunofluorescence antibody test (IFAT). Nineteen belonged to the subclass IgG1, three to IgG2 and two to IgG3. Twelve MAB's reacted only with homologous antigen. The other twelve MAB's showed cross reactions of various degrees with cystozoites of *S. gigantea*, *S. tenella*, *S. arieticanis*, *S. capracanis*, *S. miescheriana* and *S. suihominis*.

In parallel with these studies, proteins of *S. arieticanis*, *S. tenella*, *S. gigantea*, *S. capracanis*, *S. muris* and *Toxoplasma gondii* are being compared by isoelectric focusing and chromatofocusing in collaboration with the College of Veterinary Medicine, Oregon State University, USA. This has revealed pronounced differences in the protein patterns of the species examined (6).

The differing degree of specificity of the various monoclonal antibodies shows that further studies with homologous and heterologous species will be necessary before diagnostic reagents can be developed.
PRODUCTION ET CARACTÉRISATION D'ANTICORPS MONOCLONAUX POUR LE DIAGNOSTIC D'ESPÈCE DES SARCOSPORIDIES. – M. Rommel, A.M. Tenter, C. Vietmeyer et N. Mencke.

Résumé: Les auteurs ont préparé et caractérisé des anticorps monoclonaux dirigés contre les cystozoïtes de Sarcocystis muris. En utilisant les tests ELISA, Dot-ELISA et d'immunofluorescence indirecte, la réaction de douze anticorps monoclonaux ne s'est produite qu'avec des antigènes homologues. Pour les douze autres anticorps on a observé des réactions croisées, à des degrés divers, avec des cystozoïtes de S. gigantea, S. tenella, S. arieticanis, S. capracanis, S. miescheriana et S. suihominis. La comparaison, par focalisation isoélectrique, des protéines de S. arieticanis, S. tenella, S. gigantea, S. capracanis, S. muris et Toxoplasma gondii, a révélé des différences importantes.

MOTS-CLÉS : Dot-ELISA - ELISA - Immunofluorescence indirecte - Microscopie d'immunoélectrons - Sarcocystis.

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REFERENCES
