Acta Veterinaria (Beograd), Vol. 46. No. 5-6, 299-306, 1996.

UDK 619:616.993.1

# IMMUNO-CHEMICAL AND PHYSICO - CHEMICAL CHARACTERIZATION OF ANTIGENS OF *SARCOCYSTIS OVIFELIS*

SOFIJA KATIĆ - RADIVOJEVIĆ and GORDANA GAĐANSKI - OMEROVIĆ

*Faculty of Veterinary Medicine, Belgrade, Bulevar JNA 18, 11000 Beograd, Yugoslavia*

(Received, 28 Oktober 1996.)

*Our investigations were aimed at obtaining a standard antigen that would be used in the diagnostics of the animal disease - Sarcocystosis. Three antigens were isolated from S. OVIFELIS cysts in our laboratory. In order to determine the Sarcocystis species involved we used a biological test on cats as well as analysis of the ultrastructure of the wall cysts, taken from the musculature of ovine oesophagus. We ascertained that antigen I (crude cyst antigen) had 24 protein fractions (SDS PAGE, Laemly, 1970); whereas antigen II (cystozooites) had 10 protein fractions; and antigen III (cyst liquid) 13 protein fractions of approximate molecular weight 84 - 9 kDa. On the basis of SDS PAGE analysis of proteins of all three S. ovifelis antigens, 7 common protein fractions were found; whereas the following number of specific protein fractions was found each antigen: antigen I - 4, antigen II - 1, antigen III - 3. The electrophoretic mobility of proteins in agarose gel of the three isolated antigens differed, too, ranging from the alfa1 to the gamma1 zone. The antigens were examined for the presence of nucleic acids, glycoproteins and lipoproteins. (Veber and Osborn 1975.) All the three antigens are immunogenic, and analysis of the immunoelectrophoretic properties points at the specific immunochemical composition of antigen III, which crossreacts with S. suicanis antibodies. An investigation of the agglutinative properties by Hirsts test confirmed that all the three antigens could be used in serological agglutination tests.*

*Key words: Sarcocystis, antigen, diagnosis, cysts, test, validity.*

## INTRODUCTION

Sarcocystis is a newly discovered organism. It was first reported in the muscles of the domestic mouse in 1843 by Miescher, and until the early 1970's

it was regarded as innocuous. In the past 20 years, over 700 scientific publications have presented basic information on the life cycle, of the organism clinical signs of disease, diagnosis, immunity, pathogenicity and treatment (Dubey et al, 1989; Smyth, 1994). Species of *sarcocystis* are generally more host specific for their intermediate host than for their definitive host. It also infects many wild mammals, birds, colo - blooded animals and man. Endogenous sporulation of cysts ared present within the gut of the definitive host in contrast to the development of *toxplasma*. Cellular and humoral responses in infected animals indicate that *sarcocystis* species are immunogenic in intermediate hosts. Little is known of the pathogenesis of chronic sarcocystosis. It has been found that an aqueous extract of bradyzoites is toxic (sarcotoxin). Lectins are also associated with bradyzoites is toxic (sarcotoxin). Lectins are also associated with bradyzoites (Montage, 1987.). It is possible that substances released from *sarcocstis* stimulate the production of tumor - necrosis factor and that poor weight gain and low feed efficiency in sarcocystosis are regulated through growth - regulating hormones (Elsasser et al., 1986, 1988.). Because bradyzoites are obtained from the terminal stage (zoitocysts) only cross - reactive antigens are actually tested (Burgess et al., 1988; Speer et al., 1986; Speer and Burgess, 1988; Pohl et al., 1989; Katić - Radivojević, 1991). Due to the lack of a standard antigen, we undertook to determine and analyse the physico - chemical and immunochemical properties three antigens from *sarcocystis ovifelis*, isolated in our laboratories (Katić - Radivojević, 1991)

## MATERIAL AND METHODS

We isolated and purified antigens from *s. ovifelis* from muscular macrocysts in ewes. In order to determine the *sarcocystic* species involved we used a biological test on cats and analysis of the ultrastructure of the cyst wall taken from the musculature of oesophagi of sheep (Zeve, 1966). The antigen with the best agglutination ability, as determined by HIRST's test, was used for serological validation (Katić - Radivojević et al., 1991a).

The applied serological method (IFAT, Ambroise - Thoma, 1969; IHA, Lunde and Fayer, 1977) did not show any cross - reaction with *t. gondii* when the titre was higher, than 1 : 5. The diagnostic accuracy and sensitivity (Trajstman, 1979) was documented in 150 naturally infected ewes from Zaječar and Knjaževac in 1995. The end - point titre of 1/40 or higher was regarded as indicative of infection.

The concentration of proteins isolated from all three antigens was determined by Lowry et al. (1951). Lipoproteins and proteins were investigated by electrophoresis in 5% polyacrylamide gel (Weber and Osborn, 1975), Followed by staining in Sudan black dissalved in acetone - acetic acid - H20 (20 - 15 - 80 v/v). The same solvent without Sudan black, was used for destaining. The approximate molecular weight was determined by SDS - PAGE (Laemmli, 1970),

using SDS molecular weight markers (MW - SDS - 200, SIGMA, USA). Electrophoresis was run in discontinuous TRIS - HCl buffer (4 g/cl gel pH 6.8 and

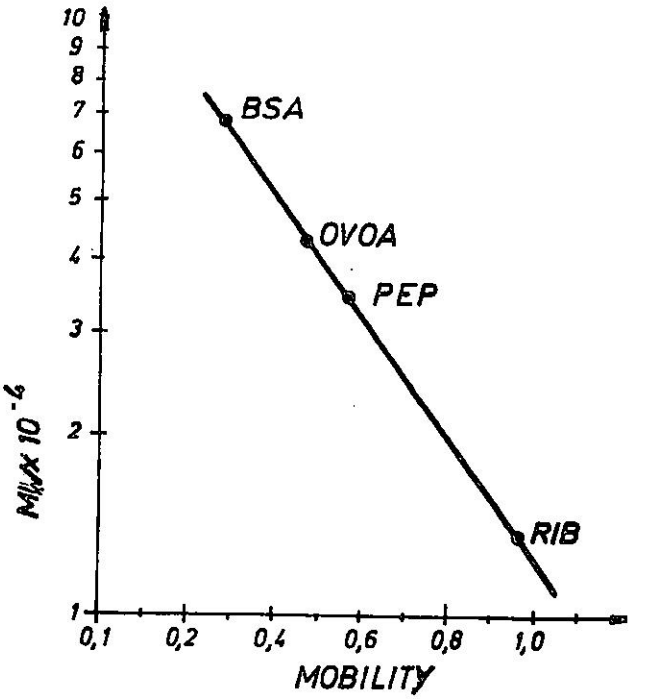


Figure 1. The values for electrophoretic mobility of proteinsof known molecular weight: Bovine albumin (BSA); ovo albumin (OVOA); persin (REP); ribonuclease (RIB).

10 g/dl pH 8.8, Figure 1). The gels were stained with Coomassie blue R 250 (Gordon, 1983) and by the PAS method in order to determine glycoproteins. Nucleic acid (DNA and RNA) was determined according to Daniel and coworkers (1980). immunochemical properties according to Ouchterlony (1958) and Habroe and Inglind (1973).

Regression analysis, correlation and a significance test were used to evaluate the results statistically (Snedecor, 1959).

Stereological investigations were performed according to Weibel (1979).

## RESULTS AND DISCUSSION

Our results showed that a calulifower - like protrusion, which characterizes the structure of wall cysts of *s. ovifelis* may be used as a criterium for separation

of species. This confirms the findings of Dubey et al., (1989) and Boch and Supperer (1992).

The accuracy, sensitivity and specificity of the tested serological methods were estinated by comparing the results obtained using direct methods (macroscopic and microscopic investigation of muscle groups) and indirect methods (IFAT and IHA test), from the same animals. Both methods have high sensitivities (0.95 -1.00), but their specificities were low (0.37 - 0.47). These results were in agreement with those of Boch and Supperer (1992) Reiter et al. (1981), and O'Donoghue and Ford (1986). A positive correlation between the results of microscopical analysis and serological tests was found (0.921). Similar results were described by O'Donoghue and Ford (1986) for ewes in South Australia. Relatively large amounts of highly purified parasites were needed to study the

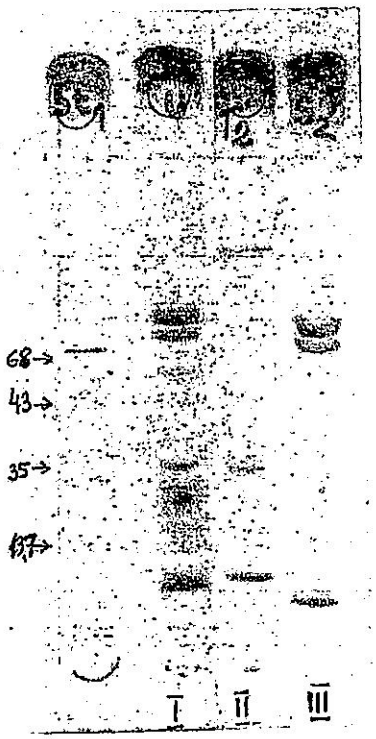


Figure 2. SDS - PAGE of the antigens I, II and III after separation of protein of solubilized antigens. Molecular weight markers: bovine serum albumin, 68 kDa; Approximate molecular weight standardsare shown at the left.

progeins and antigens of Sarcocystis spp.. *S. ovifelis* macrocysts were very suitable for the isolation of antigens for serological investigation both in ewes and other domestic animals, as suggested by Reiter and coworkers (1981) and Dubey (1989). All the three isolated antigens were immunogenic, their molecular weights being approximately 84-89 kDa (Figure 2) Table 1. SDS- PAGE analysis indicated that antigen I had 24 protein fractions in the gel length 88 mm, antigen II 10 in 89 mm, and antigen III 13 protein fractions in 89 mm of gel. Standard proteins (BSA, OA, REP, RIB) traversed 97 mm length of the gel. Approximate molecular weight standards are shown on the left (Figure 2) On the basis of their relative molecular weight 7 common protein fractions were indicated. Apart from the common fractions a specific protein fraction (of about 32 kDa) was identified in antigen III. Antigen III reacted with heterologous antibodies from the serum of a pig experimentally infected with *S. suicanis oocysts* (Figure 3). This antigen will be used for serological investigations from heterologous species. Although *Sarcocystis* species share antigens, the antibody titers were higher using antigen from homologous species of Sarcocystis than from heterologous species. Gasbarre and coworkers (1984) and O'Donoghue and Weyreter (1983). Electrophoresis using a discontinuous buffer system gave a better separation of protein fractions than with continuous buffers for the same antigens I, II and III (Katić - Radivojević, 1991). One of the characteristics of antigens I, II and III form *S. ovifelis* is the presence of nucleic acids, together with two fractions of glycoproteins and one lipoprotein fraction (Figure 4). Except for *S. cruzi,* there is little information concerning the proteins and antigens of *Sarcocystosis spp.* Protein and antigens of *S. cruzi* and *S. muris sporozoites, merozoites and bradyzoites* have been studied with SDS - PAGE and monoclonal antibody techniques by Speer and coworkers 1986, 1988; Burgess and cowarkers (1988) and Pohl and coworkers 1989; but only two proteins were present in all three parasitic stages.

Table 1. Electrophoretic mobility of proteins from S. ovifelis and M. W. of the fraction

| Name of the antigens | abbreviation | Range of the molecular weight (kDa) | Length of thr gel | Separated (kDa) values | N$^0$ of Bands |
|---|---|---|---|---|---|
| crude | antigen I | 83 – 11 | 88 | 11, 19, 23, 30, 35, 38, 42, 45, 49, 50, 52, 53, 54, 58, 63, 65, 67, 70, 71, 74, 75, 80, 83, | 24 |
| cystozoites | antigen II | 78 – 9 | 89 | 9, 12, 21, 29, 48, 53, 55, 64, 68, 78, | 10 |
| cyst liquid | antigen III | 84 – 20 | 87 | 20, 29, 32, 35, 39, 55, 60, 61, 68, 72, 78, 84, | 13 |

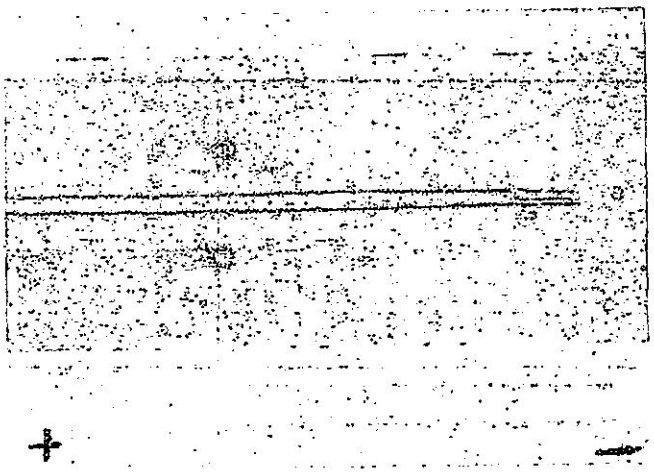

Figure 3. Immunoelectrophoresogram of antigen III, with albumin and swine serum on day 90 after infection with 200.000 sporocysts S. Suicanis
Immunoelectrophoresogram: antigen III (A III and, albumin (Al) with swine serum on day 90 after infection with 200.000 sporocysts S. Suicanis (ASS)

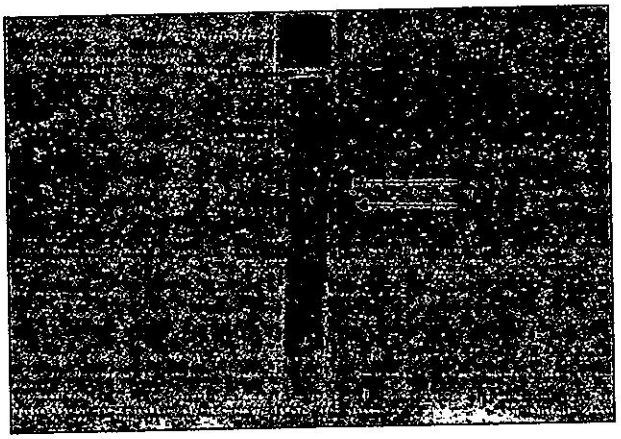

Figure 4. Antigen II From *s. ovifelis* with two fractions of glycoproteins

REFERENCES

1. *Ambroise - Thomas P.* 1969. Etude sero - Immunologique de dis parasitoses par les techniques d'immunofluorescence. *These Doctorat et Science Naturalles (Etat) Universite de Lyon.*

2. *Burgess, D. E., Speer, C. A. and Reduker, D. W.*, 1988. Identification of antigens of *Sarcocystis cruzi* sporozoites, merozoites and bradyzoites with monoclonal antibodies. *J. Parasitology, 74,* 178-199

3. *Daniel, P., Perl, D. M., Brian, W., Little, D.*, 1980. Acridin orange - nucleic acid fluorescence. Its use in diagnostic muscle biopsies. *Arch. Neurology, 37,* 641-644.

4. *Dubey, J. P., Speer, C. A. and Fayer, R.*, 1989. Sarcocystosis of animals and man. *C.R.C. Press, Inc. Boca Raton, Florida.*

5. *Elsasser, T. H., Hammond, A. C., Rumsey, T. S. and Fayer, R.*, 1986. Perturbed metabolism and hormonal profiles in calves infected with *Sarcocystis cruzi. Domest. Animal. Endocrinology, 3,* 277-298.

6. *Elsasser, T. H., Rumsey, T. S., Hammond, A. C. and Fayer, R.*, 1988. Influence of parasitism on plasma concentrations of growth hormone, somatomedin - C and somatomedin binding proteins in calve. *J. Endocrinology, 116,* 191-208.

7. *Gordon, A. H.*, 1983. Electrophoresis of proteins in polyacrylamide and starch gels, *Edited by Work E. Elsevier.*

8. *Katić - Radivojević S.*, 1991. Possibilities of diagnostics of sarcosporidiosis in domestic animal in vivo, *Dph Thesis, Faculty of Veterinary medicine, Belgrade.*

9. *Katić - Radivojević S. G. Gađanski - Omerović and Ž. Savin*, 1991a, Some diagnostic aspect of ovine sarcocystosis, *Acta Veterinaria, 41,* 197-204.

10. *Katić - Radivojević, B. Đuričić, Ž. Savin and S. Dimitrijević*, 1990. Indirect haemagglutination in the diagnostics of ovine sarcosporidiosis in sheep infected with *S. ovifelis. Vet. glasnik, 8-9,* 699-705.

11. *Laemmli U. K.*, 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T. *Nature, 227,* 680-685.

12. *Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J.*, 1951. Protein measurement with the Folin - phenol reagent, *J. Biol. Chem., 193,* 265-275.

13. *Lunde, M. N. and Fayer, R.*, 1977. Serologic test for antibody to sarcocystosis in cattle. *J. Parasitology, 63,* 222-225.

14. *Miescher, F.*, 1843. Uber eigenthumliche Schauche in den Muscless ainer Hausmaus. *Berl. Verch. Naturfosch., Ges., 5,* 198-201.

15. *Montag, T., Thietz, H. J., Brose, E., Liembetal, C., Mann W., Hiepe, F. and Coupek, J.*, 1987. The mitogenicity of extracts from *S. gigantea* macrocysts is due to lectin(s). *Parasitol Res., 74,* 112-121.

16. *Ouchterlony, O.*, 1958. Double diffusion in gel technique. *Prog. Allergy, 5,* 1 - 78.

17. *Pohl, U., Dubremetz, J. F. and Enzeroth, R.*, 1989, Characterization of the protein content of micronemes of *Sarcocytis muris* cystozoites *(Protozoa, Apicomplexa). Parasitol. Res., 75,* 199-205.

18. *Smyth, J. D.*, 1994. Tissue cyst - forming coccidia, In Introduction to animal parasitology, third edition, Cambrige University ppress.

19. *Snedecor, G.*, 1959. *Statistical Methods, Ames, Iowa.*

20. *Speer, C. A. and Burgess, D. E.*, 1988. In vitro development and antigen analysis of *Sarcocystis. Parasitol Today, 4,* 46-59.

21. *Speer, C. A., Whitmire, W. M., Reduker, D. W. and Dubey, J. P.*, 1986. In vitro cultivation of meronts of *Sarcocystis cruzi. J. Parasitology, 72,* 677-691.

22. *Weber, K. and Mary Osborn*, 1975. Proteins and Sodium Dodecyl Sulfate: Molecular Weight Determination on Polyacrylamide Gels and Related Procedure. In TheProteins, Neurath - Hill, Third edition, Academic Press, 179-223.

23. *Weibel, E. R.*, 1979. Stereological Methods, *Academic Press, London.*

24. *Zeve, H. and Herman, C. M.*, 1966. Electron microscopic study of *Sarcocystis spp. Experimental Parasitology, 18,* 338-346.

## IMUNOHEMIJSKA I FIZIČKOHEMIJSKA KARAKTERIZACIJA ANTIGENA *SARCOCYSTIS OVIFELIS*

SOFIJA KATIĆ - RADIVOJEVIĆ I GORDANA GAĐANSKI - OMEROVIĆ

SADRŽAJ

Naša ispitivanja imala su za cilj pronalaženje standardnog antigena koji bi mogao biti upotrebljen u dijagnostici oboljenja životinja - sarkocistoza. Izolovana su tri antigena iz cista *S.OVIFELIS* u našoj laboratoriji. U cilju određivanja *SARCOCYSTIS* vrsta korišćen je biološki ogled na mačkama isto tako kao i analiza ultrastrukture zida cista uzetih iz muskulature jednjaka ovaca. Ustanovili smo da antigen I (sirovi antigen cistozoita) ima 10 proteinskih frakcija; i antigen III (antigen tečnosti ciste) ima 13 protein;skih frakcija približne molekulske mase 84 - 9 kDa. Na osnovu analize SDS PAGE proteina svih antigena. *S. OVIFELIS*, ustanovili smo 7 zajedničkih frakcija, mada jeu antigenima ustanovljeno nekoliko proteinskih frakcija pojedinačno: antigen 1 - 4, antigen II - 1; antigen III - 3 specifične za svaku od njih. Elektroforetska pokretljivost proteina u gelu agaroze izolovanih antigena rasprostranjena je od $\alpha$ - $\gamma$ zone. Antigeni su bili ispitivani naprisustvo nukleinskih kiselina, glikoproteina i lipoproteina. Sva tri antigena su imunogena, i analiza imunoelektroforetskih osobina ukazuje na specifičan imunohemijski sastav antigena III, koji daje unakrsnu reakciju sa antitelima *S. SUICANIS*. Ispitivanja aglutinacionih osobina korišćenjem Hirst-ovog testa, navode na zaključak da se sva tri antigena mogu koristiti u serološkim testovima aglutinacije.