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SARCOCYSTIS GIGANTEA: STUDIES ON SPOROCYST PRODUCTION, EXCYSTATION AND VIABILITY

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ABSTRACT

Recent advances in knowledge about the sporozoan genus *Sarcocystis* (Protozoa: Apicomplexa) are reviewed and studies on the production, excystation and viability of sporocysts of *Sarcocystis gigantea*, undertaken.

Investigation of a sedimentation/floatation procedure for the mass recovery of *S. gigantea* sporocysts from cat faeces showed that the greatest yields were obtained when a proportion of faeces to floatation medium of 5% and a centrifugal force of 6000 x g for at least 5 min were used. Ninety-six percent of the sporocysts recovered were obtained from the first centrifugation in aqueous NaCl solution, specific gravity 1.2. Although neither sieving nor additional washing of homogenised samples prior to floatation significantly affected sporocyst recovery both reduced the amount of debris present. A considerable reduction in the amount of debris resulted from feeding infected cats on tinned fish rather than tinned meat. The addition of CCl_4 to the NaCl solution also improved sporocyst purity but with a marked reduction in the numbers recovered.

A technique for determining the concentration of sporocysts in faeces, using a modification of the mass recovery procedure and a haemocytometer, was developed. This was shown to be more accurate and reliable than the McMaster method for performing faecal sporocyst counts. It resulted in a mean sporocyst recovery of 75.5% and was used to obtain information about patterns of sporocyst excretion and numbers of *S. gigantea* sporocysts shed by 28 experimentally infected cats.

In all cats, sporocyst excretion commenced 10 or 11 days post-infection(PI). Peak production occurred between 13

and 22 days PI, in most instances on days 17 and 18. Peak numbers (rounded) ranged from 550 to 260,000 (mean = 53,000) sporocysts per gram of faeces or from 38,000 to 6.6. million (mean = 1.7 million) sporocysts per day.

The number of days sporocysts were shed ranged from 26 to at least 60 days PI but in 26 of the 28 infections examined, more than 80% of the total sporocyst yield was produced within 30 days of infection. The total numbers of sporocysts produced by individual cats over the patent period ranged from 164,000 to 56.6 million (mean = 12.7 million). These numbers tended to increase with increasing infective dose and to be greater in those cats receiving multiple rather than equivalent single doses. Neither the sex of the cat, nor experience of one or two previous infections, had any significant effect on the numbers of sporocysts shed.

Studies on the *in vitro* excystation of *S.gigantea* sporocysts revealed that pretreatment before exposure to trypsin and bile was an essential pre-requisite. However, in contrast to *S. tenella* and *S. capracanis*, incubation in cysteine hydrochloride under CO_2 was largely unsuccessful for excysting *S. gigantea:* of the pretreatments tested only exposure to sodium hypochlorite proved effective.

Excystation from sodium hypochlorite-pretreated *S. gigantea* sporocysts took place in trypsin and bile between temperatures of 30° and 43°C and occurred rapidly at 39°C. While the presence of bile or bile salts was essential for this process, that of trypsin was not although more sporocysts excysted in its presence than in its absence. Excystation occurred in the presence of all bile types tested but not when 'Tween 80' was substituted for bile. The highest levels of excystation were recorded when cattle or sheep bile or sodium taurocholate were used and the

lowest when chicken or pig bile were employed. Neither the concentration of sheep bile above 2.5%, nor hydrogen ion concentration (pH range 5.0 to 10.0) appeared to have any marked effect on the level of excystation obtained.

The excystation process for *S. gigantea* was similar to that described for other *Sarcocystis* species and for other coccidian genera that lack sporocyst Stieda bodies. Sporozoites escaped following the collapse of the sporocyst wall and its eventual separation into four elongated pieces.

In vivo studies on excystation of *S. gigantea* indicated that this process was, as *in vitro*, diphasic involving pretreatment and treatment phases. They also tended to support *in vitro* observations that the requirements for the excystation of *S. gigantea* and *S. tenella* sporocysts were quite different. Although the results suggested that for neither species was the pretreatment stimulus likely to be provided by conditions in the rumen alone, exposure to abomasal conditions only, induced moderate levels of excystation in both when they were subsequently treated with trypsin and bile. For *S. gigantea*, 0.25 to 4 hr abomasal exposure was most effective,for *S. tenella* 24 hours. The stimuli necessary to complete the excystation process could, apparently, be provided by 1 hr placement in the duodenum for *S. gigantea* but not for *S. tenella*.

Using *in vitro* excystation as a measure of viability, it was found that at 4°C, *S. gigantea* sporocysts survived considerably better in tap water (85% excystation after 174 days) than in either 2.5% potassium dichromate (15% excystation after 174 days) or 2% sulphuric acid (0% excystation after 5 days). Although they were able to resist 48 hr suspension at room temperature in most

laboratory reagents, disinfectants and anti-coccidial drugs tested, six (sulphuric acid, ammonia, methanol, ethanol, potassium hydroxide, sodium hydroxide, Medol*) had major sporocysticidal properties.

Further investigation with three of these, showed that sporocyst excystation was reduced from 65% to less than 10% following contact with 2.5% sulphuric acid for 1 hr or with 2% ammonia or 4% Medol for 4 hours.

Sporocysts were either killed, or their ability to excyst severely impaired, by heating to 60° and 55°C for 5 and 60 min, respectively, by exposure to ultraviolet radiation at a dose of 4000 ET, or by prolonged storage in water at 24°C. Sporocysts exposed to either constant or intermittent freezing at -18°C suffered a comparatively slow decline in excystation rate with time as did those subjected to desiccation. The duration of survival of desiccated sporocysts was inversely related to relative humidity and after 245 days at 33% RH and temperatures of 15 or 24°C, 60% of such sporocysts excysted.

Studies on the survival of *S. gigantea* sporocysts in faeces outdoors showed that, viability declined most rapidly over the summer months and suggested that they were unlikely to remain infective for more than one year.

Possible associations between the reported findings and both the epidemiology of *S. gigantea* infection and some of the previous unsuccessful or equivocal attempts to experimentally infect sheep with this species, are discussed.

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*Medol: 16% synergistic mixture of five chlorinated phenols.

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