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STUDIES ON PARASITIC PROTOZOA
OF THE GENUS *SARCOCYSTIS*

A thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy in Veterinary Science
at Massey University

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ABSTRACT

Earlier investigations into the biology of *Sarcocystis* are briefly reviewed; information reported since 1972 is reviewed in detail.

The relative efficiency of haemagglutination (HAT, macro and micro systems), complement fixation (CFT, macro and micro systems) and the indirect fluorescent antibody test (IFAT) was studied using macrocysts (*S. gigantea*) from sheep oesophagi as antigen. In the HAT, macro system titres were always higher than micro system titres. Hyperimmunised rabbits had higher titres than hyperimmunised sheep. Fifteen of 24 naturally infected sheep had negative titres. The macro CFT gave comparable results: the micro CFT was affected by persistent anticomplementary factors in sheep serum. The IFAT was both sensitive and repeatable. In all test systems, *Sarcocystis* antibody titres were minimal in infected adult sheep and in pasture-raised lambs. The value of serology in surveys of prevalence and in diagnosis of sarcocystosis is discussed.

Two types of macrocyst were found in skeletal muscle of sheep at slaughter: 'fat' cysts resembled oesophageal cysts (*S. gigantea*) grossly and in ultrastructure of the wall; 'thin' cysts (*S. medusiformis* n. sp.) were narrower and ultrastructurally distinct. The relative prevalences of the three sheep macrocysts were independent.

Fat and thin macrocysts were transmitted to cats and similarly sized sporocysts produced. *S. gigantea* sporocysts failed to infect lambs; reasons for this are discussed.

Survival of *S. gigantea* macrocysts was studied using an oxygen electrode and by cat feeding. Macrocysts were viable after 10 minutes at 52.5°C but not after 20 minutes at 55°C or 10 minutes at

60°C. Macrocysts survived 60 days at -14°C, cysts stored at 10°C for 13 days and 4°C for 30 days metabolised vigorously. Sheep meat should be exposed to 60°C for at least 20 minutes to render it non-infective for cats.

Using muscle digestion and histology, *Sarcocystis* spp. were found in (%; number examined); feral goats (28;60), red deer (30;50), wild pig (10;50), norway rat (84;50), mouse (8;50) and rabbit (16;50); none in 62 opossums and 8 wallabies. A goat species was transmitted to dogs (sporocysts $13.6 \pm 0.69 \times 9.25 \pm 0.55$), a rabbit species to cats (sporocysts $12.5 \pm 0.31 \times 9.29 \pm 0.45$) and one in rats to cats (sporocysts $10.59 \pm 0.52 \times 7.87 \pm 0.41$). Appropriate sporocysts failed to infect laboratory rats or rabbits.

A survey showed that feral cats inhabit and breed in a variety of terrains in most parts of New Zealand. The commonest foods eaten were rabbit (22% total reports), opossum (18%), sheep (16.6%) and birds (14.5%).

The development and pathogenesis of a dog-derived species was studied in goats. Doses of 5×10^6 sporocysts caused death at 18 and 19 days after infection; necropsy revealed extensive petechial haemorrhages. Schizonts occupied endothelial cells, especially in renal glomeruli. 6×10^5 sporocysts caused death at 24 and 34 days; lesser doses caused pyrexia, anaemia, anorexia and stunting. Sarcocysts were found in muscle fibres at 34 days, appeared mature at 80 days and were infective for dogs at 129 days. Changes in levels of Hb, PCV, TP, SGOT and *Sarcocystis* antibodies were shown. Four sheep given sporocysts were not infected.

The potential importance of sarcocystosis in animal production and the need for further research is discussed.

PREFACE

Sarcosporidia have been reported in the muscles of a wide variety of hosts, especially farm animals, for more than a century.

In New Zealand, slaughtered adult sheep are frequently seen to be infected with sarcocysts and carcasses have to be detained for trimming or occasionally condemned. The presence of *Sarcocystis* macrocysts in mutton adds several million dollars a year to the operating costs of the meat industry.

It has been shown experimentally that *Sarcocystis* infections in ruminants can cause mild to severe illness, even death, and abortions of pregnant animals. The importance of naturally acquired infection to farm animal production in this country is unknown.

The studies described in this thesis were contemporary with the rapid expansion in knowledge of *Sarcocystis* species after 1972 and the aim of the research was to provide basic information on these parasites in New Zealand.

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TERMINOLOGY

The following terms are used in the text:

bradyzoite, n.	Non-proliferative cell found in mature sarcocysts. Resists pepsin:HCl digestion. (syn: cystozoite, meront, spore).
cyst, n.	Abbr. sarcocyst (macro-, micro).
definitive host, (with respect to <i>Sarcocystis</i> spp.)	The host in which gamogony occurs, a carnivorous mammal, bird or reptile.
endodyogeny, n.	The formation of two daughter cells within a mother cell. See Figure 2.6
endopolgeny, n.	The synchronous formation of many daughter cells within a mother cell. See Figure 2.6.
gamogony, n.	Development of gamonts to macro- and micro-gametes and fusion to form a zygote. (syn: gametogony, sexual reproduction).
gamont, n.	Transient stage found in intestinal cells of definitive host, gives rise to macro- or microgametes.
intermediate host (with respect to <i>Sarcocystis</i> spp.)	The host in which schizogony occurs and sarcocysts develop.
macrocyst, n. (hence macrocystic, a.)	Mature sarcocyst with a diameter greater than that of a muscle fibre; may be visible without magnification.
macrogamete, n.	'Female' gamete, non-motile, intracellular. Found only in definitive host.
metrocyte, n.	Proliferative cell found in mature and immature sarcocysts, gives rise to bradyzoites, not resistant to pepsin:HCl.
microcyst, n. (hence microcystic, a.)	Mature sarcocyst with diameter less than or equal to that of a muscle fibre.
microgamete, n.	'Male' gamete, motile, flagellate, briefly extracellular (?), fuses with macrogamete to form zygote. Only in definitive host.

oocyst, n.	Resistant infective stage in life cycle of classical coccidia, but only a transient stage in <i>Sarcocystis</i> life cycle: breaks down <i>in situ</i> to release two sporocysts.
sarcocyst, n.	Long-lived, resistant cyst stage in <i>Sarcocystis</i> life cycle, intracellular in muscle. Comprises bradyzoites and metrocytes surrounded by a distinctive wall.
sarcocystosis, n.	Infection of an intermediate or definitive host, with a species of <i>Sarcocystis</i> .
sarcosporidia, n.	An old term for <i>Sarcocystis</i> species, still useful for referring collectively to sarcocysts in muscle.
schizogony, n.	Division of a schizont to form schizonts, occurs only in intermediate host. See endopolygony.
schizont, n.	Transient dividing stage in intermediate host, gives rise to schizonts by endopolygony.
schizont, n.	Product of schizogony, extra-cellular briefly, invasive, gives rise to schizont or metrocyte depending on stage of life cycle. (syn: merozoite).
spore, n.	An outdated term, replaced by bradyzoite.
sporocyst, n.	Resistant infective stage produced by definitive host, passed in faeces. Contains four sporozoites and a residuum.
sporogony, n.	Development of the cytoplasmic mass (sporont) in the oocyst to sporocysts and sporozoites. (syn: sporulation).
(The) Sporozoa, n.	All the protozoa in the Class Sporozoasida (Sub-phylum Apicomplexa).
sporozoal, a.	Referring to a member of the Class Sporozoasida, or features of such protozoa.
sporozoan, n.	A member of the Class Sporozoasida.
sporozoite, n.	Invasive cell, enclosed in sporocyst, released in excystment.

trophozoite, n.	Invasive, multiplicative stage (cf. <i>Toxoplasma</i>).
ultimate schizogony	The phase of schizogony immediately preceding the invasion of muscle.
zoite, n.	Shortened form of bradyzoite, trophozoite, sporozoite, etc.
zygote, n.	Product of fusion between macro- and microgametes, intracellular, gives rise to oocyst.