Aus der
Faculty of Veterinary Science, Veterinary Parasitology Unit
University of Melbourne, Australia
und dem
Institut für Parasitologie
der Tierärztlichen Hochschule Hannover

Investigations into the epidemiological status of hydatidosis/echinococcosis in the Falkland Islands

INAUGURAL - DISSERTATION

zur Erlangung des Grades eines

DOCTOR MEDICINAE VETERINARIAE

durch die Tierärztliche Hochschule Hannover

Vorgelegt von

Michael P Reichel

aus Langenfeld

Hannover 1994

Wissenschaftliche Betreuung:

in Hannover: Priv.-Doz. Dr. T. Schnieder

in Melbourne: Dr. R. B. Gasser

1. Gutachter: Priv.-Doz. Dr. T. Schnieder

2. Gutachter: Univ. – Prof. Dr. T. Blaha

Tag der mündlichen Prüfung: 3. Juni 1994

Dedicated to Kristin: for her patience

"ELISAs for Parasitologists: or Lies, Damned Lies and ELISAs"

(P. Venkatesan and D. Wakelin, Parasitol. Today **9**, 228-232)

Table of contents

List of figures

List of tables

List of plates

Preface

The Falkland Islands are an archipelago of some 420 islands situated between latitudes 51º and 53º South and longitudes 57º and 62º West, in the South-West corner of the South Atlantic, and thus approximately 13,000 km (or 17 flying hours) from Europe. East and West Falkland are the major inhabited Islands, separated by Falkland Sound. The Islands cover a distance of about 240 km East to West, and 140 km North to South. The most western islands are approximately 450 km from Cape Horn, Tierra del Fuego and thus from Chile and Argentina, the Falkland Islands nearest neighbours. The total land area comprises approximately 12,000 square km. The capital, Stanley, is situated at the most eastern end of East Falkland. The Falkland Islands have been a British crown colony continuously since 1833, apart from the brief interruption during the Argentine invasion in April 1982 and are, at last census, inhabited by 2050 people of mainly British descent.

The Falklands have a generally cool, oceanic climate dominated by westerly winds with a narrow temperature range. The mean temperature during summer is 10º C and 7º C during winter (June/July). The average wind speed is 16 knots, with calmer weather in the winter period. Stanley has an annual precipitation of 630 mm with less precipitation experienced on the more westerly lying isles, which may only get 400 mm per annum.

Approximately 75% of the residents live in Stanley, the rest on one of the 90-odd farms and settlements throughout the Falklands. The area outside of Stanley, i.e. all the farms and settlements is locally commonly referred to as "Camp" (from the Spanish: *Campo* = field). Agriculture has been the major employer until about 7 years ago, when fishing licences were first sold, which now provide the colony with most of its income. Up until then income has mainly been derived from the sale of wool in the United Kingdom, which is grown by the 700,000 sheep of predominantly Polwarth breed.

The government, led by the representative of the Queen, is made up of local residents, and the administration supported by many professional people which have been recruited from overseas. Thus, presently, the medical and veterinary services for example, are provided by expatriate staff from mainly the United Kingdom, but also from other Commonwealth countries, most notably New Zealand.

Permanent veterinary staffing was only possible from 1976 onwards, then still under a development grant provided by the British government, and from 1990 funded by the Falkland Islands Government. The single veterinary officer provides all the veterinary

needs of the Islands. Amongst other things, the veterinary officer provides a clinical veterinary service, is responsible for the conduct of the annual *Brucella ovis* eradication campaign and *E. granulosus* control and also for imports and exports of animals and animal products.

1. Introduction and objectives

1.1. History and epidemiology of E. granulosus in the Falkland Islands

Munro (1924), an experienced livestock officer from New Zealand, who commented on the agriculture of the Falkland Islands, and also discussed animal health problems in his report, made no mention of hydatid disease. The disease was well recognised in New Zealand at that time, and it is reasonable to assume that Munro would have discussed hydatid disease in his report, had he come across it during his stay.

The first mention of *Echinococcus* in the Falkland Islands was in the report of Dr Gibbs, then Director of Agriculture, who reported the discovery of a unilocular cyst in one of 2000 sheep slaughtered at Darwin, East Falkland in 1941 (Gibbs 1946). The next mention of hydatid cysts was in a meat inspection report from the export slaughter house that had been established at Ajax Bay, San Carlos waters, in the early 1950's, where in 14226 sheep killed in the first season, 478 (3.3%) of lungs and livers were found to contain hydatid cysts (Fletcher 1953). Fletcher also reported 1753 (12.3%) *C. tenuicollis* in the same period. In the following year, when 16901 sheep were slaughtered at the same plant, the prevalence of hydatid was reported as "common" (Rippon 1954). Rippon also reported that "in cattle *C. tenuicollis* and *Echinococcus* cysts were not uncommonly encountered in the same tissues as in sheep". The meat export trade was then abandoned as unlucrative and exact figures for the development of the disease in the Falklands sheep and dog population are not available for a number of years. A visiting British Antarctic Survey (BAS) veterinary surgeon, Mr. Godsal, makes the next mention of the disease in 1964, when it was reported as common but varied in its distribution (Miller 1965), and the first control measures were instituted with a Tapeworm Eradication (Dogs) Order (anon. 1965). Dog ownership had always required permission from the government (anon. 1949) but this new order allowed for the purging of dogs with arecoline acetarsol (Tenoban) yet did not mention any restriction on the feeding of offal.

A team of visiting British agricultural advisers arrived in 1969, and within a few weeks, the veterinary surgeon, who was part of the team, had found very high prevalences of hydatid cysts in sheep (59.3%) and cattle (61%) around the Islands, and called for control measures (McCrea 1969). Treatment of dogs with bunamidine hydrochloride (Scoloban), at three-monthly intervals, was introduced. Tenoban had fallen out of favour because of its adverse side-effects on dogs, and a second Tapeworm Eradication (Dogs) Order was instituted (anon. 1970). This order, for the first time introduced restrictions

on feeding, prohibiting the feeding of livers, lungs and hearts unless they had been stored in a dog-proof container for 28 days. The order prohibited the presence of dogs during slaughtering, and imposed a fine of £ 25.00 for offences against the order.

After a visit by Dr M. Gemmell in 1975, the Hydatids Eradication (Dogs) Order was instituted which further specified aspects of offal disposal, dog control (dogs tied up when not in use) and the maximum penalties for offences against the order increased to £500.00 (anon. 1975). From July 1977 praziquantel (Droncit®, Bayer Leverkusen, Germany) became available in the Falkland Islands and dosing at six-weekly intervals began (Whitley 1983), which has been continued until the present day. In 1981 the present, latest legislation on hydatid disease control was enacted with the Hydatid Eradication (Dogs) Order 1981 (anon. 1981, Appendix 1).

After the initial survey carried out by McCrea, which finally recorded a prevalence in sheep of 53%, the annual incidence of hydatid disease was constantly monitored at the abattoir in Port Stanley, and reduced over years with probably the most rapid reduction in the years from 1972 to 1976, when the recorded incidence fell from 47 to 13% (Figure 1). The annual incidence further decreased over the following years until it fell to 1.8%, as recorded at the abattoir in Stanley, in 1983 (Whitley 1983, Figure 1). Camp figures however remained somewhat higher than those at Stanley, mainly from 1976 to 1983, possibly because of over-recording (Figure 1).

1.2. Human cystic hydatid disease in the Falkland Islands

The first case of human hydatidosis was recorded in 1963 and further cases soon followed (Whitley 1983). During the years 1965-1975, 11 confirmed cases of human hydatidosis were noted, and the first serological survey (utilising Latex agglutination and Double diffusion Arc 5) in 1977 revealed a further 9. Five of those had had surgery for hydatid cysts previously, and only one case, a child with multiple cysts received treatment with mebendazole initially, and surgically later. The next serological survey in the early 1980's revealed 8 persons sero-positive, all but three of them, however, previously diagnosed as suffering from hydatid disease (Whitley 1983, Bleaney 1984).

In the latest human serological survey, carried out in 1988, using ELISA, 18 sera showed a positive reaction of varying degree. All cases however had previously been identified as suffering from human hydatidosis (Diggle, *pers. comm.*).

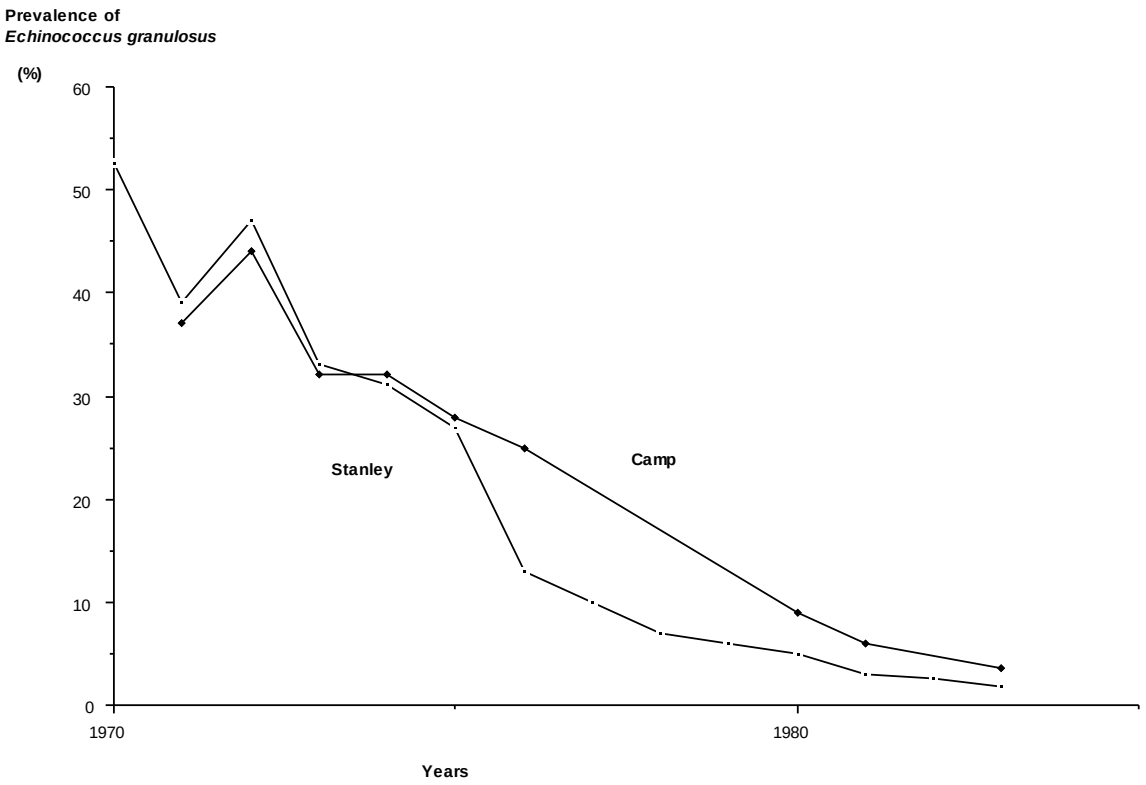


Figure 1: Prevalence of *E. granulosus* in sheep in the Falkland Islands, recorded at the Stanley abattoir and on farms (Camp) from 1970-1983 (Whitley 1983)

1.3. Aims of the work and research objectives

Many hydatid control programmes have been introduced with the ultimate goal of eradicating the adult and larval stages of *Echinococcus granulosus* from definitive and intermediate host populations, respectively. This is also the aim of the campaign in the Falkland Islands. This aim may have been difficult to achieve in the past without a major reduction in the number of dogs in the Falkland Islands, and a long and concerted effort of the farming and Stanley community alike. A powerful cesticidal drug such as praziquantel, which in other countries has been shown to break the cycle of transmission and reduce the prevalence of *E. granulosus* (Gemmell 1978), raised hopes that similar progress could be made in the Falkland Islands.

It had been stated that sheep in the Falkland Islands were generally used to a greater age than in other regions (Whitley 1983). Despite this it was predicted that, with the commencement of six-weekly praziquantel dosing of dogs in July 1977, hydatid disease would be eradicated from the Falkland Islands by 1989 (Whitley 1983).

When the author of this present study arrived in the Falkland Islands for the first time in 1989 public support for the eradication effort was strong, yet the general perception existed that hydatid disease had already been eradicated from the Falkland Islands. While no formal meat inspection was being carried out, personal observations, however, made it immediately apparent that hydatid cysts could still be found in livers and lungs of cull-for-age sheep slaughtered at the local abattoir. The metacestode of *Echinococcus granulosus* can persist in the intermediate host population for the whole life span of the infected animal, even after transmission from the definitive host population has ceased. The findings of cysts suggested though, that *E. granulosus* was not yet eradicated. No new human cases had been identified in the 1988 serological survey of the human population, and it could be concluded from this, that Cystic Hydatid Disease was being effectively controlled by the eradication programme. However, because of the absence of information on the prevalence of infection in the definitive host population, it was impossible to make any predictions as to whether the *E. granulosus* life cycle was still able to perpetuate, and thus posing a threat to human health in the Falkland Islands.

As the prevalence of hydatid cyst in sheep appeared to be rising again from 1989, it became essential to establish whether transmission still occurred. If this was in fact the case, it would then be necessary to establish the transmission patterns, the location and the causes for the breakdowns in the hydatid campaign.

The current project was designed, to monitor the intermediate (sheep) host population on a more regular basis, than had previously been the case, in order to accurately establish the prevalence of hydatid cysts. Should this surveillance of the intermediate host population demonstrate a significant level of infection and/or a rise in prevalence, further investigations would be warranted.

Surveillance of the prevalence of *E. granulosus* cysts in the intermediate host population would only give an indication of the transmission patterns which pertained at the time when those sheep became infected. As Falkland sheep were traditionally culled at 7 to 8 years of age, infection could have happened over a number of years, and a description of the transmission patterns from abattoir data alone may not accurately reflect present transmission patterns. A survey of the dog population was designed to assess whether the perpetuation of the life cycle of *E. granulosus* still occurred in the Falkland Islands in the 1990's. Since 1981 the hydatid eradication campaign had run unaltered, utilising the combination of six-weekly dosing with praziquantel, denial of access to offal and restrictions on dog movements. Arecoline purging and copro-antigen detection would only be able to detect existing infection, but with the ready availability of praziquantel to the dog owner community, a condition of the present hydatid control programme, the value of these two diagnostic tools was much diminished. The recently developed ELISA serological test system (Gasser *et al.* 1988, 1993), which detects "existing" infection, as well as "recent past" infection and exposure to worm antigen, can still be used in dog populations, which are regularly treated with praziquantel. Blood samples can be readily obtained and the ELISA technique allows them to be rapidly screened for evidence of specific anti-*E. granulosus* antibodies. This method is particularly suited for population estimates of the prevalence of *E. granulosus* infection. Therefore, this technique had the potential to detect dogs which may have been exposed to *E. granulosus*, and have a role in the transmission of hydatid disease in the Falkland Islands.

The aim of this project was to identify dogs in the Falkland Islands that were infected with or exposed to *E. granulosus* and it was therefore that the serological ELISA was chosen.

Based on the review of the literature and the conclusions made above, the current project was designed with the following aims:

1. To establish the prevalence of hydatid cysts in the Falkland Islands sheep population through abattoir surveillance.

2. To estimate the prevalence of antibodies to *Echinococcus granulosus* in the Falklands dog population.

The results gained from this study would provide insight into the present epidemiology of hydatid infection in the Falkland Islands. Furthermore, it would:

1. Identify foci of residual *E. granulosus* transmission through the identification of clusters of infected sheep or dogs.

2. Identify deficiencies of the present campaign, and

3. Enable the development of improved control strategies for future years.

2. Literature review

2.1. The genus Echinococcus Rudolphi, 1801

The Class Cestoda (kestos - tape) are divided into two Subclasses. The Cestodaria are simple tapeworms, trematode-like without scolex and without segmented body. The Eucestoda (eu - true) are the true tapeworms with a segmented, elongated body (strobila), consisting of a linear set of reproductive organs (proglottides) and a specialised anterior attachment organ (the scolex), which has four muscular suckers and a rostellum armed with a double row of hooks. Within this Class are the Orders Pseudophyllidae and Cyclophyllidae. Within the Order Cyclophyllidae is the Family of Taeniidae, and in that Family are the genus *Taenia* and *Echinococcus*. *Taenia* comprises several species of long tapeworms of veterinary and medical importance, such as *T. saginata, solium, ovis* and *T. hydatigena,* to name but a few.

The genus *Echinococcus* consists of four species: *E. multilocularis* Leukart 1863, *E. vogeli* Rausch and Bernstein 1972, *E. oligarthrus* Diesling 1863 and *E. granulosus* Batsch 1786.

The life cycle of *E. multilocularis* typically involves foxes (genera *Vulpes* and *Alopex*) as the definitive host and various rodent species as intermediate hosts for the metacestode (larva). The development of the metacestode is generally in the liver and progression to infectivity is rapid, with infective protoscoleces produced within 60 days (Rausch 1975). The domestic dog may replace the fox as the definitive host species and can be a significant source of infection to humans. In humans, the larval stage of *E. multilocularis* is the cause of alveolar hydatid disease (AHD). AHD in humans grossly resembles a malignant neoplasia, and in earlier times it was termed alveolar colloid or colloid carcinoma. Virchow (1855) recognised that the larval stage of *E. multilocularis* was the cause of this condition in humans. Primary lesions regularly occur in the liver, but metastases may be found in other organs. Because of the infiltrative nature of the lesions they are difficult to remove surgically and often fatal (Vuitton 1990). The parasite is reported from arctic to moderate regions, with Tunisia it's most southern reported range, but it does not appear to occur in the Southern Hemisphere (Schantz *et al.* 1991). Prevalences in foxes can be high, with as many as 71% reported infected in Siberia (Gubanov 1964), and between 13.5 to 55 per cent of foxes in Southern Germany and Northern Switzerland (Vogel 1961, Zeyhle 1982, Gottstein *et al.* 1991). Recent studies have also shown that the range of *E. multilocularis* distribution includes Northern and Central regions of Germany (Frank 1987, Fessler 1990). In North America

the cycle involves the red fox and coyote. Surveys have shown from 13.7% to 67% of foxes and coyotes in some states of the US infected (Rausch and Richards 1971, Storandt and Kazacos 1993).

E. vogeli has been described from the bush dog (*Speothos venaticus*) from Ecuador (Rausch and Bernstein 1972) and is known to occur elsewhere in South and Central America, but its distribution and host range are not well understood. The domestic dog can replace the bush dog as a definitive host. The intermediate host is typically the paca *Cuniculus paca* L, where the larval stage usually develops in the liver (Rausch *et al.* 1981). The larval stage of the cestode is the cause of polycystic hydatid disease in humans (D'Alessandro *et al.* 1979). The prevalence for the larval stage in the paca in Colombia ranges from 19 to 23 per cent (D'Allessandro *et al.* 1981).

E. oligarthrus, first described from a cougar, *Felis concolor L.* in Brazil, occurs in various felid species, and the larval stage was found in an agouti, *Dasyprocta* spp. In the intermediate host metacestodes may be found in subcutaneous muscle as well as in the liver and other organs (Cameron 1926, Sousa and Thatcher 1969, D'Allessandro *et al.* 1981). It is the only *Echinococcus* whose larval stage has not been found in humans. In southern Argentina seven out of 46 Geoffroy's cats have been reported infected (Schantz and Colli 1973).

Echinococcus granulosus is the cause of cystic hydatid disease (CHD) in humans, but normally involves in its life cycle canids as definitive hosts and ungulates as intermediate hosts. Two biological forms of *Echinococcus granulosus* can be distinguished: a Northern form, where the metacestode occurs exclusively in the Family Cervidae (deer, reindeer, elk and others) and the adult in the wolf, *Canis lupus* L. The other is the European form, where the cycle is perpetuated by domesticated animals, with the dog the definitive host of any significance, which becomes infected from scavenging metacestode infested offal. Some authors consider the Northern form to have been the original life cycle, from deer to wolf, which, after the domestication of animals, about 10000 years ago spread to involve sheep and dogs. With colonisation in the past 400 years this domestic dog-sheep cycle has spread to other countries, including the New World, Australia and New Zealand (Rausch 1986, Gemmell 1990).

E. granulosus infections have been reported from many countries (Schwabe 1986), with major foci of human and animal infections still in China (Craig *et al.* 1991), East Africa (Macpherson 1983), and South America, ie. Argentina (Schantz *et al.* 1973), Chile (Wilhelm 1953) and Uruguay (Purriel *et al.* 1973). *E. granulosus* has also been reported

from Australia (Dew 1926, McConnell and Green 1979) and the United Kingdom (Blamire *et al.* 1980, Lloyd *et al.* 1991) and up until recently in New Zealand (Gemmell 1961, 1978, anon. 1992).

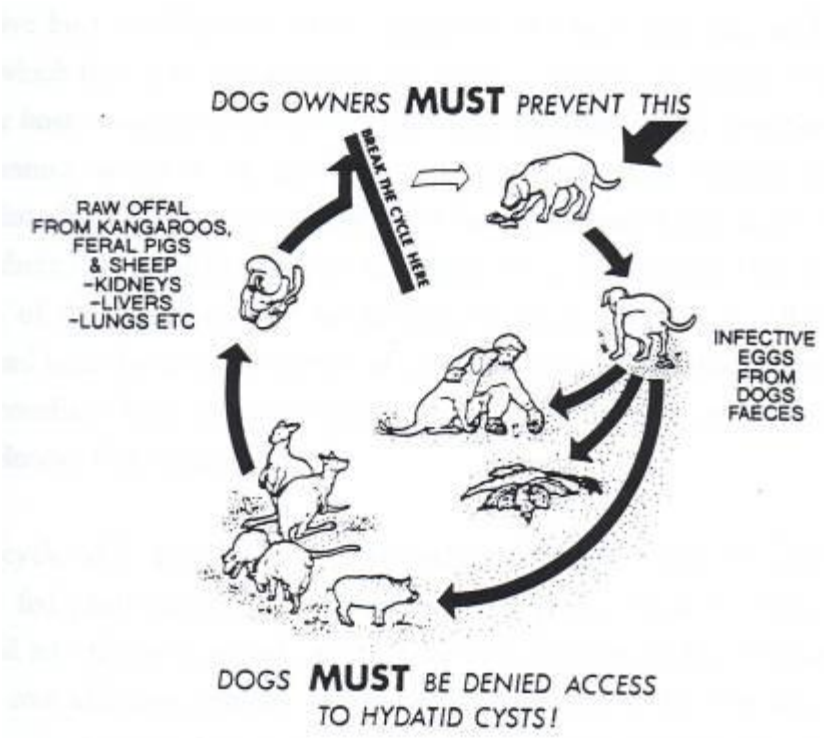


Figure 2: Life cycle of *Echinococcus granulosus* (after Thompson *et al.* 1993)

2.2. Biology and life cycle of E. granulosus

Echinococcus granulosus requires two mammalian hosts for completion of its life cycle, a definitive host in which the adult develops in the small intestine, and an intermediate host in which the cystic metacestode (*E. cysticus*) develops, usually in the viscera. The definitive host is always a carnivore which becomes infected by ingesting protoscoleces, the immature scoleces of the next generation of adult worms (Figure 2). The intermediate host is often an ungulate, but macropod marsupials have also been shown to be infected in Australia (Durie and Riek 1952, Thompson 1986). The time from infection of the definitive host to the first shedding of eggs is called the pre-patent period, and with the commencement of egg shedding the infection has become patent. In the intermediate host the metacestode reproduces through asexual multiplication of protoscoleces (Thompson 1986).

The life cycle of *E. granulosus* was reproduced experimentally by von Siebold (1853), when he fed protoscoleces of *Echinococcus veterinorum* from cattle to dogs which developed into the adult worm. At that time two forms of hydatid disease were thought to exist, one afflicting animals and the other humans (*E. hominis* syn. *altriciparien*s) (Küchenmeister 1855). However, a few years after von Siebold's experiments came the realisation that the disease in animals and in humans was caused by the same parasite (Naunyn 1863).

An adult *Echinococcus granulosus* worm in the definitive host is only 2 to 11 millimetres long and possesses three, sometimes up to seven proglottides, which sets it apart from the major genus in the same Family, *Taenia*. The latter can grow several metres in length and consist of several thousand proglottides (Thompson 1986).

2.2.1. Development in the definitive host

Protoscolex

The apical region of the protoscolex (rostellum, suckers and hooks) is invaginated within the basal region of the protoscolex tegument (Marchiondo and Andersen 1983) which protects the protoscolex until it evaginates under the influence of gastric and intestinal fluids of the dog's intestine. The precise stimulus for evagination is not clearly understood, but protoscoleces are sensitive to environmental changes and evaginate in response to changes in temperature and osmotic pressure and to agitation (De Rycke

1968). Bile is not essential but the rate of evagination is increased in the presence of bile (Smyth 1967). Aerobic conditions, however, are essential for evagination (Smyth 1969). Up to 86.5% of protoscoleces may evaginate within 6 hours of ingestion, but complete evagination may take up to 3 days (Thompson 1977). Protoscoleces are very active initially, utilising their glycogen reserves but these are used up within three hours (Smyth 1967). This appears to be a necessary prerequisite to establishment in the dog intestine. Juvenile worms, still evaginating can already be found deep within the crypts of Lieberkühn by six hours post infection (p.i.) (Thompson 1977). Developing worms attach by grasping plugs of intestinal tissue with their suckers which act as anchors and prevent the worm from being swept away (Smyth *et al.* 1969, Thompson *et al.* 1979, Thompson and Eckert 1983). Activity then declines for eight days but recommences thereafter (Thompson 1975).

Adult worm

Mature *E. granulosus* are mostly found in the anterior quarter of the small intestine, whereas *E. multilocularis* occurs in the posterior regions. Once the worm reaches maturity it appears to remain in a particular region of the small intestine (Thompson *et al.* 1979, Thompson and Eckert 1983). From day 20 to 35 p.i. mature worms are found with the rostellum deeply embedded in the crypts of Lieberkühn with the apical rostellar region fully extended, the hooks penetrating the mucosal epithelium to some degree and the suckers grasping the epithelium at the base of the villi (Smyth *et al.* 1969, Thompson *et al.* 1979, Thompson and Eckert 1983). Other taeniids achieve only a relatively small degree of penetration (Featherston 1971, Beveridge and Rickard 1975).

The invasion of the crypts of Lieberkühn coincides with the beginning of secretory activity from specialised tegumental cells (rostellar gland) with release of material into the interface between parasite and host tissue (Smyth *et al.* 1969, Thompson *et al.* 1979, Thompson and Eckert 1983). It has proposed that this secretion either firmly attaches the worm in the intestine, preparing them for the shedding of proglottides, as unattached worms do not shed their terminal proglottides (Thompson and Eckert 1982). It has also been suggested that it protects the worm through the inhibition of host digestive enzymes or the host immune mechanisms (Thompson *et al.* 1979).

Echinococcus infection rarely elicits a pathological response in the definitive host, and the hooks cause little damage (Thompson *et al.* 1979). Thus, the adult worm may reside in the intestine for periods of up to two years (Schantz 1982).

The mature *Echinococcus* is hermaphroditic and capable of self-insemination (Smyth and Smyth 1969). The onset of egg production varies between species and sometimes even between strains. For *E. granulosus*, it ranges from 34 to 58 days, with the shorter interval noted for the specific cattle strain in Switzerland (Thompson and Eckert 1982, Thompson *et al.* 1984) and the Tasmanian sheep-dog strain (Kumaratilake *et al.* 1983). The number of eggs produced may range from 100 to 1500 per proglottis (Arundel 1972, Rausch 1975, Thompson and Eckert 1982, Wachira *et al.* 1991), but it is not clear how often proglottides are shed. Some estimates assume that proglottides are produced and detached every 7 - 14 days (Smyth 1964, Schantz 1982), others observed a delay of between four to six weeks between first and subsequent appearance of eggs with *E. granulosus* (Yamashita *et al.* 1956).

2.2.2. Development in the intermediate host

Egg/Oncosphere

Echinococcus eggs are spherical to ellipsoid in shape and range in size from 30 µm x 50 µm to 22 µm x 44 µm. They are indistinguishable under a light microscope from the eggs of other taeniid cestodes, all consisting of an outer envelope (egg capsule), an inner envelope (embryophore and granular layer), the oncospheral membrane and the oncosphere (Lethbridge 1980, Swiderski 1982). The outer envelope is stripped from the egg before it is excreted in dog faeces. The eggs are fully embryonated and infective when released from the proglottis, although some evidence suggests that they may require a short period of maturation (Willis *et al.* 1981).

Proglottides with eggs may migrate from the site of defecation (Mattoff and Kolev 1964) but can be dispersed further by up to 80 metres in 10 days according to one report (Gemmell and Johnstone 1976). Deplazes and Eckert (1988a) also provided evidence that proglottis deposition was independent from faecal deposition, and in fact for *T. hydatigena*, at least, may comprise the bulk of the proglottis excretion. Other studies provided evidence that flies can transmit eggs of *Echinococcus* (Schiller 1954, Heinz and Brauns 1955).

Taeniid eggs survived better at lower temperatures, up to 294 days at 7°C, after which they had retained their invasiveness, yet failed to form cysts in the intermediate host (Gemmell 1977). Even after 478 days at 4°C some eggs retained their infectivity (Schaefer 1984). Deplazes and Eckert (1988b) stored *T. hydatigena* eggs at 4°C for 180

days after which they remained viable, yet after storage at -28°C for 180 days only 4 % remained viable, and none after 270 days. Other authors report that they may survive freezing at -50°C (Colli and Williams 1972), but only tolerated elevated temperatures for short periods (Schaefer 1984). Desiccation killed eggs even after only one or two days (Laws 1968), exposure to sunlight within a few hours (Wachira *et al.* 1991).

Ingested by a suitable intermediate host, eggs hatch in the stomach and small intestine. This involves the disaggregation of the embryophoric blocks in the stomach and the activation of the oncosphere (Lethbridge 1980), possibly under the influence of bile (Smyth 1969). The activated oncosphere penetrates the tips of the villi in the jejunal and upper ileal regions of the small intestine (Heath 1971) reaching the lamina propria within 30-120 minutes (Lethbridge 1980). Penetration of the epithelium may be aided by enzymatic activity or be purely by a mechanical mechanism, involving hook and body movements. Subsequently, oncospheres are transported via lymph or blood vessel to their predilection sites, which may vary between species. In humans, ungulate and macropod hosts these are mainly lung and liver. However, in these hosts other organs may also be affected, including the central nervous, musculoskeletal, genito-urinary, endocrine and circulatory system (Bickers 1970, Schantz 1972a, Perez-Gomez *et al.* 1973, Duran *et al.* 1978, Bähr 1981, Chin 1981).

Metacestode (Larva)

Postoncospheral development proceeds at the predilection sites towards the formation of a metacestode after a series of reorganisational events during the first fortnight of infection. This includes hook degeneration, muscular atrophy, cellular proliferation, cavity formation and development of both germinal and laminated layers (Rausch 1954, Sakamoto and Sugimura 1970, Heath and Lawrence 1976). These processes are induced by the growth and division of germinal cells (Slais 1973).

The fully developed metacestode of *E. granulosus* is typically unilocular, subspherical in shape and is fluid-filled. The cyst consists of an inner germinal layer supported by an elastic, acellular laminated layer of variable thickness, surrounded by a host-produced fibrous adventitial layer. Growth is by concentric enlargement. From the germinal layer arise brood capsules which vacuolate and become stalked. The laminated layer contains polysaccharide proteins and stains periodic acid-Schiff (PAS) positive (Kilejian *et al.* 1961, Kilejian and Schwabe 1971), which provides useful diagnostic identification.

The rate of development of the cyst depends on a number of "strain" and host specific factors, but is slow in *E. granulosus*, with a growth rate varying between 1 to 5 cm per annum. Brood capsule formation is usually not expected before 10 to 48 months in sheep (Heath 1973), but has been reported as early as 5 months in New Zealand (anon. 1988).

2.2.3. Strain differences

Traditionally "strains" of *Echinococcus granulosus* have been described on the basis of homogeneity within them and biological differences between them, ie. the range of definitive and intermediate hosts a strain appeared to be involving, differences in morphology, adult development to maturity. Domestic strains, usually involve domesticated ungulates (sheep and cattle) as intermediate hosts, and dogs as definitive hosts. Sylvatic biological "strains" utilise commonly foxes (*Vulpes*) as definitive hosts, and ungulates, but also marsupial macropods at the intermediate host level.

Recent studies at the molecular level have shown that biological, phenotypic, as well as genetic differences exist between the Australian mainland and the Tasmanian strain but the genetic difference is not substantial (Lymbery and Thompson 1988, Thompson and Lymbery 1988). Other authors find differences non-existent at mitochondrial DNA level (Bowles *et al.* 1992). This, latter, extensive study identified 7 distinct genetic "strains" of *E. granulosus* by analysis of the mitochondrial DNA in 49 isolates from 18 different countries and 11 intermediate hosts. Another study supports the view that the "strains" on the Australian mainland are indistinguishable at the DNA level and may therefore be genetically the same as the common sheep variant (Hope *et al.* 1992). Thompson and Lymbery (1990a) conclude that while studies at the molecular level have great value in new approaches to the prophylaxis and diagnosis of parasitic disease, they should not be the sole means of characterising a parasite population. Genetic results, biology, morphology and epidemiology should be interpreted in combination in a comprehensive approach.

In the definitive host, host specificity is relatively high, and almost exclusively restricted to canids. There is, however, one strain of *Echinococcus granulosus* which utilises the lion (Graber and Thal 1980). The red fox (*Vulpes vulpes*) is a suitable definitive host (Thompson 1983) and in Australia the sylvatic strain of *E. granulosus* utilises both the domestic dog (*Canis f. familiaris*) and the dingo (*Canis familiaris dingo*) (Kumaratilake and Thompson 1984). More worms establish and develop more rapidly in the dingo (Thompson and Kumaratilake 1985). In the United Kingdom sheepdogs are more

susceptible to infection with the UK strain of *E. granulosus* than beagles (Walters and Clarkson 1980).

In the United Kingdom, strains are determined by the host from which the parasite originates. A sheep and a horse strain can be distinguished, each showing different growth and other biological and biochemical characteristics (Williams and Sweatman 1963, Smyth and Davies 1974, Thompson and Smyth 1975, Smyth 1977, 1982, Thompson 1979, Thompson and Kumaratilake 1982). Equine "strains" are also described from other countries and all possess uniform morphological characteristics (Kumaratilake *et al.* 1986)

In Australia, three main strains have been recognised, namely the Australian domestic sheep/dog strain, the Tasmanian sheep/dog strain and the macropod marsupial/dingo (dog) sylvatic strain. Cattle are commonly found to be infected, however these cysts are often found to be sterile or degenerated (Durie and Riek 1952, Gemmell 1957, 1959, Jackson and Arundel 1971, Baldock *et al.* 1985, Morrison *et al.* 1988). In the Tasmanian strain the period of maturation to egg production is more rapid (up to 7 days shorter) compared to that of the mainland strain, which is very similar to the UK and New Zealand sheepdog strains (Kumaratilake *et al.* 1983, Thompson and Lymbery 1990b).

In Kenya, at least three (biological) strains appear to occur, of which the sheep strain seems to be the major source of infection to man (McManus 1981, Macpherson and McManus 1982, Macpherson 1983, McManus and Macpherson 1984). In other parts of Africa, a sylvatic cycle involves lions and a number of intermediate hosts (Macpherson *et al.* 1983), which in the Central African Republic includes the warthog (Graber and Thal 1980).

In Switzerland, the most important cycle exists for *E. granulosus* infections between dog and cattle, with 0.2% of cattle estimated to be infected with cysts (Eckert 1970, 1981). The Swiss cattle isolates are quite distinct from other isolates of domestic origin in Europe and Australia (Thompson *et al.* 1984). In particular, the early rate of maturation of the adult worm is remarkable, which leads to egg production as early as 34 days, and the almost exclusive location of cysts in the lungs of cattle.

In Germany, the life cycle of *E. granulosus* appears to be similar to the situation described in Switzerland, with a dog/cattle cycle being the most predominant form (Hörchner *et al.* 1986, Worbes 1986). The pre-patent period of this particular strain is equally short, as in the Swiss isolates, with 0.2 to 0.6% of cattle bearing cysts in the

lungs. A recent study has identified a distinct pig "strain" from Eastern European pigs and evidence that they are morphologically and genetically distinct from cattle, sheep, camel and horse strains (Eckert *et al.* 1993).

Table 1: Biological "strains" of *Echinococcus granulosus* in various countries

Country	Strain	References
Australia	Dog-sheep	Thompson and Kumaratilake 1982
	Canid-macropod	Durie and Rick 1952, Thompson and Kumaratilake 1982
	Dog-sheep (Tasm)	Thompson and Kumaratilake 1982
Central African Republic	Lion-Warthog	Graber and Thal 1980
Germany	Dog-cattle	Hörchner *et al.* 1986 Worbes 1986
Kenya	Canid-sheep/goat -human	McManus and Macpherson 1984
	Canid-cattle	McManus and Macpherson 1984
	Canid-camel	McManus and Macpherson 1984
New Zealand	Dog-sheep	Thompson and Lymbery 1990b
Poland	Dog-pig	Eckert *et al.* 1993
Switzerland	Dog-cattle	Eckert 1970, 1981 Thompson *et al.* 1984
United Kingdom	Dog-sheep	Thompson and Smyth 1975 Walters and Clarkson 1980
	Dog-horse	Williams and Sweatman 1963 Thompson and Smyth 1975

2.3. Immunology of E. granulosus infection in dogs

2.3.1. General aspects of immunology in the dog

Seven antigenetically distinct immunoglobulins are found in dog serum, namely IgG1 (Sg1), IgG2a (g2a), IgG2b (g2b), IgG2c (g2c), IgA (Int Sg1), IgM (19Sgm) and IgE (Johnson and Vaughan 1967, Johnson *et al.* 1967, Reynolds and Johnson 1970, Whitacre and Land 1980). Dog IgA is dimeric in serum and in mucosal secretions (Tizard 1992). Pedigree dogs have significantly lower serum IgG2ab levels (Johnson *et al.* 1967) than have mongrel dogs IgG2d (Reynolds and Johnson 1970). Immunoglobulins are secreted locally in the gut (Reynolds and Johnson 1970) and been found contained in cells of the intestinal mucosa with a ration of IgA-producing cells to IgM and IgG producing cells of approximately 2:1:1. The great majority of plasma cells is found in the proximal regions of the small intestine (Hart 1979).

Young puppies show mainly IgM producing plasma cells in the *lamina propria* about two weeks after birth, and by three weeks IgA producing cells can be found. After four to five weeks, plasma cells producing IgM, IgA and IgG are found. IgM producing cells can first be found in the duodenum, then jejunum and finally in the large intestine. Similar gradual onsets occur later for IgG and IgA producing cells. Cells producing IgG1 and IgG2ab are found in smaller numbers. The number of IgM producing cells varies according to location, and more cells are found in duodenum and jejunum, respectively than there are in the ileum (Willard *et al.* 1978). Immunoglobulins are also secreted onto the mucosal surface of the upper gastrointestinal and the respiratory tract and are contained in the saliva of the dog. Saliva contains IgA, IgG1, IgG2ab, IgG2c and IgM, with IgA being the most predominant (Vaerman *et al.* 1970). In the stomach, and in the intestine, IgG1 and IgG2ab producing plasma cells are found less frequently than are those which produce IgG2c (Vaerman *et al.* 1970).

Antibody and cell mediated effector mechanisms are all involved in the defence of the organism against infectious pathogens such as bacteria, viruses and fungi (von Fellenberg 1978, Bienenstock and Befus 1980, Schwartz and Kehoe 1983, Newby and Stokes 1984). Antibody mediated immune responses which involve the Fc regions of Ig's (mainly IgG and IgM) also include complement proteins (Chakravarti and Kristensen 1986). Cell mediated responses associated with macrophages, lymphocytes or granulocytes include destruction of the pathogen which can be bound to membranes or intracellular (von Fellenberg 1978, Roitt 1984).

In parasitic infections eosinophilia and elevated IgE levels are often encountered, yet other mechanisms such as complement-dependent antibody mediated toxicity, macrophage or neutrophil antibody dependent toxicity and non-specific inflammatory effects may also play significant roles (Schwartz and Kehoe 1983).

2.3.2. Specific aspects of dog immunology relating to *E. granulosus*

Innate resistance

Non-specific innate protective mechanisms have been suggested for the susceptibility of dogs against infection with *E. granulosus*. Age of dogs and dose rate of protoscoleces do not appear to influence whether a dog becomes infected, or determine the number of worms which establish. At dose rates ranging from 10 to 175000 protoscoleces per dog, the distribution was overdispersed, with the majority of dogs harbouring only very few worms (or none at all), and only few dogs being highly susceptible (Gemmell *et al.* 1986). Other authors have recently described a litter and host-sex dependent susceptibility to infection with *Echinococcus granulosus*, with more growth and fecundity in female dogs than in males, and more and longer adult parasites developing in two out of three litter of dogs (Barriga and Al-Khalidi 1991).

Acquired immunity

Evidence for acquired immunity in dogs against infections with *E. granulosus* is conflicting. Several studies have shown that vaccination of dogs with homologous somatic or excretory/secretory antigens reduced the susceptibility to subsequent challenges. Number of established worms were affected, their growth and fecundity (Gemmell 1976, Rickard 1983). Gemmell *et al.* (1986) showed that most dogs acquired a certain amount of resistance to subsequent challenges when infected several (nine) times with protoscoleces over up to three years, with infections regularly terminated with arecoline hydrobromide. A significant correlation existed between number of challenges given to a dog and the number of worms that established in subsequent infections. Estimates suggested that half of the dogs acquired a degree of immunity to infection after six challenges, and 99% after 12. Parenteral injections of oncospheres of *E. granulosus*, *Taenia hydatigena*, *T. multiceps*, *T. ovis*, *T. serialis* could induce a transient immune response against *E. granulosus* infections which affected the number of worms which became established, their growth and oogenesis. Heterologous oral

vaccination with oncospheres, however did not induce resistance to subsequent challenges.

Antigens prepared from protoscoleces, germinal membranes or cyst fluid derived from hydatid cysts were prepared for subcutaneous and intramuscular injection in dogs (Turner *et al.* 1933). A high degree of protection was afforded when challenged six to 15 days after the last injection, which was reflected in reduced numbers of established worms per dog, compared with a control group of unvaccinated dogs. Gemmell (1962) vaccinated dogs intramuscularly with a preparation from protoscoleces and subsequently challenged these dogs and a control group with 50000 protoscoleces. Although only 4 mature dogs could be considered as being resistant to infection (less than 500 worms per dog at necropsy), numbers of worms that became established were generally lower in the vaccinated group than in the unvaccinated control group. Oral vaccination with irradiated *E. granulosus* protoscoleces resulted in reduced numbers of worms established after experimental challenge (Movsesijan *et al.* 1968, Movsesijan and Mladenovic 1971). Adult worm antigen injected intramuscularly reduced the worm burden after challenge and stunted worm growth (Gemmell 1962) and suggested also that the degree of immunity conferred by worm antigen was greater than that achieved by vaccination with protoscoleces material.

Antibody responses

Chordi *et al.* (1962) first reported the detection of anti-*E. granulosus* serum antibodies in dogs with *E. granulosus* infection by betonite flotation (BFT) and complement fixation (CFT) test systems using hydatid cyst fluid antigen. While not all dogs were positive to each test when they were infected, sera from dogs which did not harbour helminths gave negative test results. Some dogs that had infections with other helminths but *E. granulosus* gave positive reactions as well. Singh and Dhar (1988) reported the detection of specific anti-*E. granulosus* antibodies in sera from puppies with experimental *E. granulosus* infection using indirect fluorescence antibody test (IFAT).

Intradermal inoculation of hydatid cyst fluid after prior infection with protoscoleces resulted in immediate-type hypersensitivity (Williams and Pérez Esandi 1971), while none of the uninfected controls showed this reactions.

Monospecific *E. granulosus* infection produces specific systemic antibody responses against the parasite which can be measured by enzyme-linked immunosorbent assay

(ELISA) (Jenkins and Rickard 1985, 1986). Dogs were raised helminth free prior to the experiment (Jenkins and Rickard 1984) and infected with between 50 to 250000 protoscoleces of sheep origin. Sera were collected from each dog every five days for 75. Patency of infection was confirmed by the detection of *E. granulosus* eggs in the faeces. Anti- *E. granulosus* antibodies (IgG) were detected in ELISA utilising protoscolex antigen (somatic or excretory/secretory). The antibody titres peaked at about 15 days, decreased by day 35 and increased again during the patency phase of infection. Sera of uninfected dogs gave negative reactions in ELISA testing throughout the experiment. Antibodies in these dogs did also not cross-react with antigens prepared from *Taenia* spp. Sera from dogs with monospecific infections with *Taenia* spp. did also not cross-react with *E. granulosus* protoscolex antigen. Here, antibody titres correlated with the length of time an adult worm persisted in the definitive host, but not with the number of worms (Heath *et al.* 1985). Antibodies remained detectable for several (on average four to six) weeks even after the removal of the worm through anthelmintic treatment (Heath *et al.* 1985, 1988).

On this basis, ELISA systems have been developed further and they allow the detection of natural *Echinococcus* infections in canids. These have been evaluated in various countries (Gasser *et al.* 1988, 1992, 1993, Jenkins *et al.* 1990, 1991, Gottstein *et al.* 1991). The dynamics of antibody levels after purgation of *E. granulosus* infections in dogs, however, and, in particular, the relative levels of the different immunoglobulin classes have yet to be followed in detail in controlled experiments (Gasser *et al.* 1993). Serological test results do not correlate with actual *E. granulosus* worm numbers in individual canids (Gasser *et al.* 1990a). This is in accordance with studies on the use of serology for the diagnosis of *E. multilocularis* infection (Gottstein 1985). In areas endemic for *E. multilocularis*, prevalence of serum antibodies against Em2-antigen in fox populations reflected previous or present exposure to *E. multilocularis* antigen, but did not always allow reliably the detection of actual intestinal infection. However, on a population basis prevalence of serum antibodies was correlated with parasitological prevalence of *E. multilocularis* (Gottstein *et al.* 1991). This demonstrates the usefulness of serological testing for definitive host populations.

2.4. Aspects of E. granulosus infection in humans

The first one to be credited with possibly describing cystic hydatid disease (CHD) in man, and the sequelae, was Hippocrates who described that "the liver is filled with water cyst and bursts, in this case the belly is filled with water and the patient dies" (as quoted in Nott 1979). It was only in the 19th century that it was realised that the cysts in animals were caused by a parasite and that it was the same which caused hydatid cysts in humans (von Siebold 1853, Virchow 1855). This zoonotic aspect of the disease has meant that it tended to be reported more often from areas and countries, where humans and sheep (cattle) and dogs were working and living in a close relationship. However, modern behavioural patterns, like recreational hunting, may give rise to artificial transmission patterns, and spread the infection beyond the boundaries of a traditional (domestic) cycle of transmission. It is suggested from Western Australia that hunter's dogs may feed on macropod marsupial and pig offal containing cysts, and then spread in turn eggs, which amplifies the natural transmission pattern, possibly leading to the establishment of a sustainable sylvatic cycle on the outskirts of a metropolitan area (Thompson *et al.* 1993).

2.4.1. Infection

When humans accidentally ingest the eggs of *Echinococcus granulosus*, cysts tend to develop with preference in two sites, the liver and lungs. The majority of lesions is found in the liver (52 to 77%), with slightly lesser frequency in the lungs (8.5 to 44%) (Schantz 1972b). Lesions can, however, occur in virtually any other organ of the body, and it has been suggested that the eventual site of cyst development is dependent on whether the oncospheres penetrate the venule of the intestinal villus, or its lymphatic lacteal and then be distributed throughout the whole body (Heath 1971). About 1 to 2.5% of cysts are believed to occur in the skeletal system, leading to bone destruction (Duran *et al.* 1978). Kidney cysts may be found in 2% of human cases (Kirkland 1966), but can also be seen in endocrine tissues (Golematis 1983). Rarely the brain can be affected (0.2 to 2.4%) in hydatid patients, with the victims often presenting with convulsions and hemiparesis, in association with headaches and vomiting (Begg *et al.* 1957, Schantz 1972a).

2.4.2. Growth rates

Growth rates in man can vary, and a slow rate of development has been reported of indigenous people in Alaska (Wilson *et al.* 1968), while in the Turkana district of Kenya

cysts of 5 to 10 cm diameter are observed in children of three and five years of age (Macpherson 1983). Varying, and in particular very slow growth rates may explain the reported extremely long apparent incubation period (to detection) of 53 years (Spruance 1974).

2.4.3. Clinical disease

Clinical disease is often the result of the space-occupying nature of the growing cyst and the pressure it exerts onto the host tissue. A cellular immune response is mostly present, frequently resulting in pericystic fibrosis (Schwabe *et al.* 1959), but more serious complications include multiple cysts and ruptured cysts which often are accompanied by anaphylactoid reactions, sometimes leading to death (Jakubowski and Barnard 1971).

2.4.4. Prevalence

In any given country clinically diagnosed infections represent only a fraction of the existing human infection. This is for one a reflection of the long period between infection and recognition of the disease, and partly because the disease is often under- or misdiagnosed. In Uruguay and the Rio Negro region of Argentina this relationship has been estimated. Every year 17.7 new surgical cases per 100000 persons were being notified in Uruguay, yet mass radiography results suggested that the figure may be as high as 150 per 100000. In Argentina the annual incidence was 143 cases per 100,000, yet 460 cases per 100,000 were estimated from radiographs (Purriel *et al.* 1973, Schantz *et al.* 1973). Annual surgical incidence for the North of Kenya is estimated to be 220 per 100,000 (Macpherson 1983).

2.4.5. Ethnic and cultural factors

Ethnic and cultural factors also predispose to hydatid disease. Maori in New Zealand were 6.4 times more likely to be infected than their European counterparts, presumably because of their closer relationship to dogs, which they treat as pets. In pre-European times the dogs sometimes also served as a food source, and it is thought that the intimate human-dog relationship which was part of Maori life that pre-disposed to infection (Burridge and Schwabe 1977a, b, Burridge *et al.* 1977a, b).

In the Turkana district the population maintains a large number of dogs and sleep with them to keep warm (Schwabe 1984). Women keep dogs as "wet nurses", to lick infants clean after they defecate or vomit, thus increasing the likelihood of transmission (French *et al.* 1982, Macpherson 1983). Women are reported to be three times more often infected than men, because of their closer and longer contact with dogs (Watson-Jones and Macpherson 1988).

2.4.6. Diagnostic techniques

During the course of infection the intermediate host will be exposed to antigens from the establishing metacestode of *E. granulosus*. These may be associated with the oncosphere, or the juvenile and/or mature hydatid cyst. Experimentally both, species and stage-specific antigens have been demonstrated in somatic and excretory/secretory molecules.

Antigens associated with the mature metacestode, and in particular the hydatid cyst fluid have been extensively studied. Several have been described in hydatid cyst fluid and tissues, and the immune response of the host against these in immunoelectrophoresis became the basis of diagnostic tests for CHD. The two major polymeric lipoprotein antigens which occur, antigen 5 or designated arc 5 for the precipitin band and antigen 4 or also called antigen B (Chordi and Kagan 1965, Oriol *et al.* 1971) which were later seen to consist of two sub-units (Shepherd and McManus 1987) and antigen B, with three sub-units of 12, 16 and 23kDa (Leggatt *et al.* 1992). Antigen 5 remains the most widely used diagnostic test for CHD, relying on the demonstration of an immunoprecipitin band (Arc 5) in immunoelectrophoresis (IEP) (Capron *et al.* 1967) while the 12kDa molecule of antigen B has been evaluated. In a recent study it was shown to have a high sensitivity for *E. granulosus* infection in human sera (90.9%), but cross-reacted with 40% of sera from humans infected with *E. multilocularis* (Leggatt *et al.* 1992).

The appearance of an immediate hypersensitivity reaction after intradermal injection of hydatid fluid antigen has been widely used for a long time and is known as the Casoni test. Complement fixation has been also widely used in the past, however this test has been shown to lack a satisfactory degree of sensitivity and specificity (Kagan 1968).

Indirect haemagglutination (Garabedian *et al.* 1957) and the latex agglutination test (Varela-Diaz *et al.* 1975, Rickard 1984) for the detection of human hydatid infection are credited to be very sensitive and also show a high degree of specificity.

Indirect fluorescent antibody test and now ELISA (Matossian *et al.* 1979, Rickard *et al.* 1984) have been used recently with a high degree of sensitivity for diagnosis.

Most of these diagnostic tools are probably only of use in the detection of patients that have as yet not shown signs of clinical disease, e.g. in large-scale screens, or where a tentative diagnosis has been made. As titres remain high for long periods of time, even after the cysts have been removed, these tests are of no direct use in post-operative screening. Radiological and ultrasonographical examination, Computer axial tomography are used to demonstrate the cysts (Morris 1981). Traditionally treatment is still by surgery, ie. complete cyst removal, although conservative treatment with chemotherapeutics, mainly benzimidazole derivatives, is been attempted with varying results (Bryceson *et al.* 1982, Morris *et al.* 1983). In cases where cysts are found to be inoperable, albendazole treatment has been used with success in a number of cases and shown to be forcing cyst development into regression and even caused a collapse of the cysts (Okelo 1986).

2.5. Epidemiology of E. granulosus infection

2.5.1. Epidemiology of *E. granulosus*

Küchenmeister, in 1852, was the first to determine the life cycle of a taeniid cestode by feeding larval stages of *Taenia pisiformis* that then transformed into adult worms. The life cycle of *Echinococcus granulosus* was first described by von Siebold in 1853. Initially the parasite was thought to exist in two forms (Küchenmeister 1855), one afflicting animals the other humans. Not much later came the realisation that the parasite which had been observed in humans and animals was one and the same, and that the disease, in fact, was transferable from animals to humans (Naunyn 1863). In the following years several authors described hydatid disease in humans as the consequence of infection with the larval stage of *Echinococcus granulosus* (Thomas 1881, Madelung 1885, MacDonald 1888). However it was only in this century that surveys in several countries revealed the extent of the infection in animals and that concerted efforts were made to control the further spread of the disease (Gemmell 1957, 1959, 1961, Schantz and Schwabe 1969, Matossian *et al.* 1977). A knowledge of the epidemiology of the life cycle and the biology of *Echinococcus granulosus* was necessary to be able to devise appropriate control programmes that could limit the further perpetuation of the transmission between the two host stages.

There are cycles of *Echinococcus* without any involvement of domestic animals, like the dingo-wallaby life cycle of Australia (Durie and Riek 1952, Gemmell 1959). Domestic animals and man, for that matter, feature in this cycle only as accidental hosts, when they invade the ecological niche this cycle occupies (Thompson *et al.* 1987). In the domestic cycle intermediate hosts may be sheep, cattle, pigs, goats, camels, horses and other species, dependent on the strain. Wildlife and domestic cycles may inter-connect, with both cycles co-existing in the same environment (Rausch 1967, Fay 1973).

In areas where the disease is endemic, enough eggs are available in the environment to ensure that sufficient intermediate hosts ingest them, and develop into the metacestode. The intermediate host has to survive sufficiently long, to ensure that the metacestode reaches maturity and is then available to the carnivore population. Thus the metacestode of *Echinococcus vogeli* matures quicker, in less than two to four months (Rausch 1967) than the cyst of *E. granulosus* before they produce protoscoleces, because in the former the intermediate host population has a much shorter lifespan. *E. granulosus* metacestodes can afford the rather longer maturation to protoscoleces formation, as the intermediate host, sheep or macropod marsupials, generally live longer.

2.5.2. Aspects of the epidemiology in the definitive host

When dogs were experimentally infected with protoscoleces, ranging from 10 to 175000, adult worm counts in dogs that were fed equal amounts varied. 5.6% of all dogs failed to become infected, and of the ones that did 17% had less than 100 worms, 11% between 100 and 1000 and 72% in excess of 1000 adult worms (Gemmell *et al.* 1986). In a field study in Victoria, Australia, only 3% of dogs (out of 1062 examined) had adult *E. granulosus* worms in their purge, and only 2 out of the 24 with worms in the purge had more than 500 (Jackson and Arundel 1971); 270 dogs, however failed to provide a purge in this study. In another study 18 to 24% had more than 500 worms (Gemmell 1957).

A study of the distribution of worms in dogs fed protoscoleces of sheep origin (17500, 87500 and 175000) found that the distribution was over-dispersed. Age or sex of the dog did not modify the distribution and the proportion of protoscoleces to developing adult worms remained roughly constant at 20 to 1 (Gemmell *et al.* 1986).

Immunity may develop in dogs which are subjected to repeated challenge with protoscoleces and reduce that proportion even further and increase the percentage of dogs that remain refractory to subsequent challenge. However, egg output by the adult worms appears to be undiminished (Gemmell 1976, Rickard 1983).

2.5.3. Egg population and egg dispersal

Not all adult worms resident in the dog's intestine shed proglottides (and eggs) every day. While the number of eggs has been estimated at 100 to 1500 per proglottis (Rausch and Schiller 1956, Arundel 1972, Rausch 1975, Thompson and Eckert 1982), a dog found to harbour 12767 worms in its intestine had a daily maximum egg output of only 71,000 eggs (Sweatman and Williams 1963).

Contractions of the proglottis may aid egg expulsion (Fay 1973) and proglottides may migrate several centimetres from the faecal mass (Matoff and Kolev 1964). Dispersal through other means however is probably more important and eggs have been found to spread up to 80 metres from the initial site of deposition in 10 days (Gemmell and Johnstone 1976) or up to 175 metres (Lawson and Gemmell 1983). For *T. hydatigena* and *T. ovis* egg dispersal up to 10 km and over up to 30000 ha has been suggested (Gemmell 1978). Work by Deplazes and Eckert (1988a) on taeniid eggs suggests that

egg deposition is not coupled to faecal deposition and that in fact the majority of proglottides, and therefore eggs, are voided from the intestine independent from defecation. This may also be true for echinococcal egg deposition and the movements of dogs thus may have much greater importance than their defecatory habits.

That flies can transmit eggs of *Echinococcus* has been firmly established, with eggs being found in the gut of several species of blowflies, with several hundred eggs sometimes found in individual flies (Schiller 1954, Heinz and Brauns 1955, Lawson and Gemmell 1985). Birds have also been suggested as vectors for taeniid eggs, mainly through circumstantial evidence (Torgerson *et al.* 1992).

2.5.4. Egg survival

Eggs of *E. granulosus* survive lower temperatures better than higher ones, and may even be frozen for extended periods of time, without loosing their infectivity for the intermediate host (Déve 1910, Schiller 1955, Gemmell 1977). Desiccation however, ie. an environment with a relative humidity of 60 to 80 per cent resulted in them dying off (Laws 1968).

When eggs of *T. hydatigena* were deposited on pasture for 0, 3, and six months the proportion of embryos which developed into cysticerci decreased from 60% over 25% to 15% (Gemmell and McNamara 1976). If *E. granulosus* eggs are exposed to sunlight and high temperatures together, as can be encountered in the Turkana district of Kenya, survival may not exceed two hours (Wachira *et al.* 1991).

2.5.5. Aspects of the epidemiology in the intermediate host populations

Not every egg that is ingested by a suitable intermediate host develops into a cyst. When sheep were fed a varying number of eggs only one out of 250 eggs developed into a cyst in one report (Gemmell *et al.* 1986). As the number of eggs which a sheep was exposed to increased, the more likely it was to become infected, ie. 38% of sheep which were fed 25 eggs remained uninfected, yet only 7% of sheep which had been fed 2500 eggs did not develop an infection. Once established cysts rarely died and the life span of the cyst was the same as that of its host. Ingested egg numbers were correlated to the number of viable cysts (ie. with protoscoleces) that developed (Gemmell *et al.* 1986).

There is some evidence that the ingestion of eggs stimulates immunity in the intermediate host which may protect against subsequent further challenge. This may require high egg numbers at immunisation (50000 to 100000 eggs) and this immunity is lost again, after 3 to 9 months (Sweatman *et al.* 1963, Gemmell and Johnstone 1981).

In estimations of the infection pressure of *E. granulosus* in an endemic area, the age-specific infection rate in sheep infected with *E. granulosus* was compared with that of sheep infected with cysts of *Taenia hydatigena* (*Cysticercus tenuicollis*). While all susceptible sheep were infected with the larval stages of *T. hydatigena* within 6 months and the age-specific prevalence rates plateaued out, age-specific infection rates continued to increase in sheep infected with *E. granulosus* until 7 years of age (Gemmell 1961) indicating a much lower infection pressure for *E. granulosus* than for *T. hydatigena.*

2.6. Examples of hydatid control and surveillance programmes

When it was realised that *Echinococcus granulosus* infection caused cystic hydatid disease (CHD) and the life cycle of the parasite understood, efforts were made to control the disease. Because of the zoonotic nature of the disease, emphasis was placed on control, ie. the breaking of the life cycle of the parasite, with the aim of totally eradicating it from intermediate and definitive host populations. This is probably an achievable aim, where only a domestic ie. sheep-dog life cycle of the parasite exists. In countries where a wild-life cycle of *E. granulosus* exists or wildlife and domestic cycle(s) exist together, a more realistic programme may restrict itself to surveillance of the epidemiological situation, which collects data on the prevalence of the disease in the respective host populations, and intervenes with the appropriate measures to curb a further expansion of the geographic range.

Control programmes have been instituted in a variety of countries, notably and probably first in Iceland, but also New Zealand and Tasmania, the latter two countries with a high sheep-dog-man ratio (Krabbe 1864, Gemmell 1958, Begg 1961, Beard 1969). As part of the control programmes, research was undertaken into the epidemiology of *E. granulosus* infection and the understanding of the biology of the cestode, its reproductive rate and factors that led to its dispersal in the environment. The control programmes in Iceland, New Zealand and to a degree in Tasmania have been the most successful, in that they eradicated the parasite from the countries in the case of the two former, and drastically reduced it's prevalence in the case of the latter (Beard 1973, Bramble 1974, anon. 1986, anon. 1992).

2.6.1. Iceland

At the start of the campaign in Iceland the number of CHD amongst the human population was 2 to 2.5% in 1864 according to Krabbe (1864), but may have been as high as 22% (Dungal 1957). This conclusion was drawn from the prevalence of hydatid cysts in the cohort of Icelanders, born in 1861-1870, and autopsied in Reykjavik between 1932 and 1950. *E. granulosus* was frequently seen in a large proportion of Icelandic dogs (28%) (Krabbe 1864). A publication which was distributed by the government in Icelandic to the whole population, summarised the knowledge of the day on the epidemiology of *E. granulosus*. While it is now 130 years old, this booklet already contained the important rules which were to be found in many of the later control programmes: denial of access to offal for all dogs, a limitation on the number of

dogs and the purging of dogs with arecoline, in an attempt to clear the adult stage from the dog's intestine. Apart from this educational component, the Icelandic campaign introduced a tax on all dogs, not needed for work with sheep, a dog census and required the destruction of all cyst material. One region in Iceland prohibited farm home killing all together, while others tried to restrict it by banning the sale of farm-killed meat. Dogs are not allowed to roam, and in some towns they are not allowed at all. Although there were still a few hydatid cysts being detected in Iceland up to 1970, it would appear that these measures combined were largely successful in driving *E. granulosus* to extinction in Iceland, with the last known human case dating back to 1960 (Beard 1973).

A similar programme was instituted in Cyprus with a particular emphasis on the reduction in numbers of dogs thereby eliminating some of the definitive hosts, in an effort to control hydatid infection (Polydorou 1976).

2.6.2. New Zealand

In the Styx field trial (which was to be the model of the national hydatid campaign), initiated in a valley in the South Island of New Zealand, dog owners were requested not to feed their dogs raw offal and submit them to a treatment with arecoline hydrobromide every three months. In the first 9 years of the trial, taeniid cysts (both *T. hydatigena and E. granulosus*) declined from 80% to 40%, *E. granulosus* however more so than *T. hydatigena*. In later years the control campaign was backed up by a strong educational effort, directed at dog owners, which emphasised the necessity of denial of access to offal for the dogs, and included surveillance in the dog population through the examination of the arecoline purges for evidence of *E. granulosus* infection. Thereafter *E. granulosus* prevalence declined towards zero (Gemmell 1978).

Control efforts in New Zealand have later been reliant on the six-weekly dosing with praziquantel (Droncit®, Bayer, Leverkusen, Germany), and a selected owner policy (anon. 1988) which allowed farmers with dog-proof home-killing facilities and city dog owners to lengthen the intervals between treatments. Abattoir findings of hydatid cysts were traced back to the farm of origin (anon. 1988). Surveillance of prevalence rates in sheep in New Zealand abattoirs has seen a continued decline in numbers of hydatid cysts and it has been stated that *E. granulosus* had been eradicated from New Zealand by 1991 (anon. 1992).

2.6.3. Tasmania

The first move towards the control of hydatid disease in Tasmania was made in 1960 but the control campaign really only started in 1964 (anon. 1986, Gemmell 1990) after the 10 years period to 1962 had seen 537 people undergo surgery for hydatid cysts (Bramble 1974). Local hydatid eradication committees, based on the organisation of the hydatid eradication campaign in New Zealand, were set up all over the state and encouraged community members to take positive steps towards curbing the spread of the parasite. The campaign relied initially on arecoline hydrobromide surveillance (which began in September 1964) and an educational campaign, which explained the life cycle of *E. granulosus*, and emphasised the denial of access to offal for dogs. Around 12% of dogs were shown to harbour *E. granulosus* in 1964-65, but over the 10 years to 1974 this was gradually reduced to 0.67%, and further to 1985-86 to 0.03% (Bramble 1974, anon. 1986). Prevalence in sheep decreased from 52.2% in 1966-67 to 7% in 1973-74, and further to 0.2% in 1985-86 (Bramble 1974, anon. 1986). New surgical human cases declined from 19 in 1966 to 6 in 1973 (Bramble 1974), and there were still 2 new cases in 1985. These two, however, belonged to the over 50's age group, thus most likely representing infection dating back a number of years (anon. 1986).

Tasmania introduced a mobile laboratory which could examine arecoline purges in the field, putting much emphasis on the educational value of demonstrating to owners of infected dogs the adult *E. granulosus* in the purge. Purging from 1967 concentrated on rural dogs as it had been shown that urban dogs had only one hundredth of the prevalence of *E. granulosus* infection of rural dogs (anon. 1986). From 1969 on restrictions on the movements of infected dogs were introduced which was extended in 1971 to quarantine infected dogs and later (from 1975) infected properties, e.g. properties with a level of infection in sheep of more than 20% or the state average (Meldrum and McConnell 1968, Bramble 1975, McConnell and Green 1979, anon. 1986). The introduction of quarantine measure has been seen as a significant step in the hydatid campaign of Tasmania, as non-complying farmers could be forced to comply with effective hydatid control measures.

Praziquantel has not been made available to the general public in Tasmania until the second half of the 1980's (anon. 1986) because it was felt that it would interfere with the other control measures, and allow dog owners to continue to feed offal, and treat the dog just prior to an arecoline purge. Even now it is not actively promoted as an effective cesticidal drug in Tasmania (Obendorf, *pers. comm.*).

Abattoir surveillance continues in Tasmania and there are still 17 properties quarantined (Obendorf, *pers. comm.*) with provisions in the legislation for compulsory acquisition of stock by the government if it is deemed likely to be infected.

Serological testing of dogs has been used recently in Tasmania on infected properties, and also on King Island in combination with arecoline purging and was carried out after the detection of hydatid cysts in sheep, cattle or goats. Sero-positive dogs were detected in all outbreaks investigated, but *E. granulosus* could only be demonstrated in one dog (Obendorf, *pers. comm*.).

Importations of animals from other Australian states into Tasmania require a statement of absence of hydatid disease on the property of origin for sheep, goats and cattle, yet there are no import requirements relating to hydatid disease for the importation of dogs (anon. 1986).

2.7. Techniques for the diagnosis of E. granulosus infection in the definitive host

Gemmell (1975) had stated in his review of the Falkland Islands hydatid control programme that before any control system was initiated, it would be advisable that a survey of the level of infection in the definitive host population be undertaken. This should have established the prevalence prior to any control efforts and would have allowed to assess the campaign success over the years. While one small survey was undertaken, preceding the commencement of praziquantel treatment (Whitley 1983), a comprehensive or representative survey was never attempted. As that survey was based on the coprological examination of faecal samples, identifying presumably eggs, it failed to selectively identify *E. granulosus* infections.

2.7.1. Traditional techniques for the diagnosis of *E. granulosus* infection - coprological examination and arecoline purging

Classical coprological examination for taeniid eggs cannot distinguish *Echinococcus* eggs from other taeniid eggs at the light microscope level (Thienpont *et al.* 1986). After the first appearance of eggs in the faeces of infected dogs at the end of the pre-patent period, there may be a delay of 4-6 weeks before any subsequent release of eggs (Yamashita *et al.* 1956). If only a few adult worms were present in the intestine the faecal material available for examination may not contain eggs at all given sampling times and a definitive host could be falsely diagnosed as being uninfected. With high numbers of adult worms at different stages of development, the likelihood of detection (the sensitivity of the technique) would presumably increase.

Autopsies will identify *E. granulosus* infections in the dog's intestine, but may still not detect all infections with a low number of adult worms, due to the small worms being missed during the examination. This method is obviously also unsuitable for large-scale surveillance, as only very few dog owners would submit their animals for such a procedure willingly.

Coprological examination after purging with the parasympathomimetic drug arecoline hydrobromide relies on the detection of the adult worms in the duodenal purge. This test system has a low sensitivity as arecoline may fail to expel all resident worms, especially if worm burdens are low. In one study, the sensitivity has been estimated as being as low as 10%, ie. more than 90% of adult *E. granulosus* infections could go unnoticed if this

test system was employed (Wachira *et al.* 1990). *E. granulosus* infections were suspected to go to full patency and persist for up to two years, despite arecoline purging at three-monthly intervals. This led to it being regarded a very unreliable tool for the elimination of *E. granulosus* infection (Gemmell 1973).

Arecoline hydrobromide has also been known to have adverse side-effects which may cause treated dogs to vomit soon after treatment, or even to collapse and die, and limit its use for large scale surveys. In one survey where arecoline hydrobromide was used on more than 1000 dogs, in excess of 40% of dogs showed vomiting, and 25.4% failed to purge at all. Five dogs collapsed and had to be given atropine. Arecoline hydrobromide should also not be used in pregnant bitches or in debilitated animals (Jackson and Arundel 1971). In surveys in low-endemic areas for *E. granulosus* infection, this may result in a greater number of adverse reactions against arecoline than infected dogs could be identified and result in a rapid decline in the level of cooperation from dog owners.

The availability of praziquantel, a highly effective cesticidal drug, to dog owners also diminishes the usefulness of arecoline surveys in such areas where dog owners, fearing the social stigma of having their dog identified as being infected or the possible legal implications, treat their dog with praziquantel just prior to the arecoline dose, resulting in negative purges.

2.7.2. Copro-antigen detection

Parasitic antigen is shed from the developing adult cestode into the intestinal tract of the definitive host, and some voided with the faeces. These are termed copro-antigens of *E. granulosus*. Copro-antigens were first described by Babos (1962) who used precipitation reactions with hyperimmune rabbit serum to detect antigens of *E. granulosus* in faeces of infected dogs. Recently, novel techniques have shown that copro-antigens may be detected during the pre-patent period of infection, as early as 10 days post infection (Deplazes *et al.* 1992) and then persist during patency, and for up to five days after an infection has been terminated by cesticidal treatment (Deplazes *et al.* 1990). An ELISA using polyclonal antibodies raised against excretory/secretory antigen of adult *E. granulosus* was highly specific (98%) and sensitive (87%), for dogs and dingoes with a burden of over 200 adult *E. granulosus*. Low worm numbers in the dog's or dingoes intestine, ie. below 200 adult worms, escaped reliable detection by copro-antigen ELISA. The test detected only 12.5% of infected dogs if the number of adult worms was less than 200 with the overall sensitivity falling to 56% (dog) and 46% (dingo),

respectively (Deplazes *et al.* 1992). Other workers reported a copro-antigen immuno-assay against *E. granulosus* with a sensitivity of 87.5 to 88.5%, and with 96.5% specificity, which was largely unaffected by the number of adult worms resident in the gut (Allan *et al.* 1992).

Further work has focused on the diagnosis of infection with intestinal cestodes in animals and humans (Allan and Craig 1989, Allan *et al.* 1990, Craig *et al.* (submitted), Deplazes *et al.* 1990, 1991). For the rapid diagnosis in the field situation, dipstick ELISAs for the detection of *Taenia*-specific copro-antigens in humans have been developed and reported a high specificity of 99.9% and a sensitivity of 75.6% (Allan *et al.* 1993).

In endemic and especially in hyper-endemic areas, where transmission occurs at reasonably frequent intervals, a survey for *E. granulosus* infection relying on copro-antigen detection would quickly identify all foci of patent and pre-patent infections. With a sensitivity of the test system of reportedly 85% to 87.5% (for infections exceeding 200 worms per dog), not all individual dogs infected at any one time would be identified during the survey, but the numbers detected would allow an accurate assessment of the epidemiological situation (Allan *et al.* 1992, Deplazes *et al.* 1992). With copro-antigen techniques only those dogs are detected which are infected with the adult worm (or have been just recently), and are thus taking an active part in the epidemiology of the disease.

In a low-endemic area, ie. with low prevalence in the intermediate host population (and in the definitive population as well), particularly when regular treatment with an effective cesticidal drug is employed, the number of definitive hosts which are carrying a pre-patent or patent infection at any given point in time, will be low.

To allow a complete picture of the epidemiological situation to develop, several copro-antigen surveys would have to be employed from time to time to highlight all foci in which transmission was still happening.

2.7.3. Serological techniques

After the early studies of Turner *et al.* (1935) which demonstrated skin sensitising antibodies in *E. granulosus*-infected dogs, and further work by Williams and Pérez Esandi (1971), serum antibodies against *E. granulosus* were demonstrated by various other workers (Movsesijan and Mladenovic 1971, Singh and Dhar 1988). More recent

work has used the enzyme-linked immunosorbent assay (ELISA) for the detection of specific anti-*E. granulosus* antibodies (Jenkins and Rickard 1985, 1986, Gasser *et al.* 1988, 1990a). These techniques have been further developed and show great promise in the detection of *E. granulosus* infection in the definitive host. They detect specific antibodies which have been raised by the definitive host, in response to *E. granulosus* infection. Specific antibody titres can be first detected after one week (IgA, IgG, IgM classes), IgG antibodies peak on day 21 after an experimental infection, IgAs after 3-5 weeks and IgEs and IgMs after 1-2 weeks and 5 weeks, respectively (Gasser *et al.* 1993). In a recent study in several countries, the overall sensitivity of an ELISA system using three antibody classes (IgG, IgA and IgE) usually ranged from 72% to 84%, with a specificity of 97% (Gasser *et al.* 1993). This degree of sensitivity compares favourably with other available diagnostic techniques, such as arecoline and copro-antigen detection (Gemmell 1973, Wachira *et al.* 1991, Allan *et al.* 1992, Deplazes *et al.* 1992). Some of the dogs which are currently infected fail to be detected by the test and thus reduce the sensitivity. This has been noted by several workers (Gasser *et al.* 1989, 1990b, Gottstein *et al.* 1991) and possible explanations include, "molecular mimicry" or "masking" of the parasite antigen by host molecules (Rickard 1983), immunosuppression or inactivation of the immune mechanisms by parasite molecules (Mitchell 1987) or differences in the genetic make-up related to the histocompatibility complex of the dog (Wakelin 1985).

In contrast to other techniques, such as arecoline purging and copro-antigen assays, serological antibody detection has the advantage of being able to detect current and past infection, or exposure to *E. granulosus* antigen. Although not always reliable in the detection of infection in an individual dog, serological techniques are useful for rapidly screening populations of definitive hosts. Infected populations can be accurately distinguished from uninfected ones and the technique used to monitor the progress of control campaigns. It has been shown that levels of antibodies detected by serology in dog populations reflect the parasitological prevalence in endemic areas.

In populations where praziquantel is used at regular intervals to control cystic hydatid disease or is otherwise available to dog owners, no other test is currently available which would be able to screen populations of dogs reliably. It is particularly useful in control programmes in the identification of dog owners that may still allow their dogs access to cyst material, but treat the dogs prior to a test (Heath *et al.* 1985).The educational value of serological results can not be overstated, as dog owners which have become complacent and allowed their dogs to become infected, and have relied on praziquantel to treat any possible *E. granulosus* infection, can be identified and confronted with the results.

3. Materials and Methods

3.1. Bleeding of dogs

Blood (5ml) was taken from 908 dogs in the Falkland Islands. All dogs over the age of 6 months at the time of the visit were bled between October 1992 and October 1993 by venopuncture from the cephalic vein using 5ml syringes (Monoject). Bloods were allowed to clot in sterile glass tubes and stored at +4°C until they were transported to the laboratory. There they were centrifuged at 2000g for 10 minutes and then stored at -20°C.

After the first ELISA testing of sera, 12 dogs that were identified as possibly infected with or exposed to *E. granulosus* were re-bled, purged with arecoline hydrobromide and treated with praziquantel (Droncit®) by oral or subcutaneous administration. At the same time a further 61 dogs, which were present in those locations were also bled a second time. All owners were asked to complete a questionnaire (Appendix 2).

All sera were transported to the University of Melbourne, School of Veterinary Science, Werribee on dried ice and stored there at -70°C.

The blood samples were taken during routine visits to farms as part of the *Brucella ovis* eradication programme or veterinary clinical calls to the remaining settlements. The blood collections and arecoline purges were done with less than 48 hours notification to the owner.

3.2. Arecoline hydrobromide purges

Dogs were given orally 3mg of arecoline hydrobromide per kilogram bodyweight, and half of the original dose was repeated if no purge resulted within an hour. Purged faeces were examined with a magnifying glass, and the duodenal purge transferred to a sample bottle. In the laboratory, the duodenal purge was examined after re-suspension of the semi-solid purge in 0.01M mouse-tonicity phosphate buffered saline (MT-PBS) pH 7.2 under a stereo microscope and manual manipulation. After sieving through a 150mm sieve, the resultant filtrate was centrifuged at 2000g for five minutes and re-examined as above. The supernatant was discarded and the pellet re-suspended in 10 times its volume of 1.3 specific gravity $NaNO_3$, inverted three times and allowed to settle for five minutes. A cover slip was placed on the uppermost layer for 20 minutes and examined

under the microscope at 40x magnification. If eggs were found a McMaster egg count was carried out (Thienpont *et al.* 1986).

3.3. Enzyme-linked immunosorbent assay (ELISA)

Flat bottomed 96 well-microtitration plates (Greiner and Sons, Nürtingen, Germany) were sensitised overnight at +4°C with 50µl, per well, of protoscolex somatic antigen of sheep origin, diluted to 15µg per millilitre protein in 50mM bicarbonate-carbonate buffer, pH 9.6 (BCB, Sigma). The antigen was prepared as described by Gasser *et al.* (1988). Briefly, protoscoleces were derived from hydatid cysts in livers and lungs of sheep slaughtered at a New South Wales abattoir (Blayney). Cysts were packed in ice and transported, overnight, to the laboratory. Protoscoleces were aspirated aseptically in cyst fluid, sifted through a double layer of gauze and allowed to settle for 10 minutes. The supernatant was discarded and the protoscoleces were washed three times in ten times their volume of mouse-tonicity phosphate-buffered-saline (MT-PBS) (Sigma Immuno Chemicals (IC), St. Louis, USA, P-4417) by centrifugation at 600g for 5 minutes, the supernatant aspirated and the pelleted protoscoleces stored at -70°C. The thawed protoscoleces were suspended in 2 x volume 20 mM Tris/HCL, pH 8.0, containing 1% sodium deoxycholate (DOC, BDH Chemicals, Poole, United Kingdom), 2mM phenylmethylsulfonyl fluoride (Sigma) and 100 IU/ml aprotinin (Trasylol®, Bayer Pharmaceutical Co., Botany, NSW) and disrupted by sonication in a 150W ultrasonic disintegrator (MSE, Crawley, Sussex, England) on ice for 15 minutes (15µm peak to peak, 5 sec on, 10 sec. off). The sonicate was centrifuged at 10000g (4°C) for 15 minutes and the supernatant dialysed for 48 hours against 5 changes of 500 x vol. MT-PBS. The protein concentration was determined as described by Bradford (1976) and the antigen stored in aliquots of 100 µl at -70°C.

After sensitisation for 12 hours, the microtitration plates were emptied and washed three times with MT-PBS containing 0.3% v/v polyoxyethylene-sorbitan monolaurate (Tween 20) (Sigma IC, P-1379) (MT-PBS-T). The wells were blocked with 100µl per well of MT-PBS-T containing 5% w/v skim milk powder for 30 minutes at room temperature, and sera were then tested at 1/25 dilution.

Sera from four Australian sheep dogs were used as controls. Two sera from dogs, mono-specifically infected for 75 days with 100000 *E. granulosus* protoscoleces were used as positive controls, and sera from two dogs that were raised helminth-free and known never to have been infected with *E. granulosus* as negative control (Table 5). The two

positive reference sera, two negative control sera, and no antigen control and no serum controls were included on each plate. Plates were then incubated at room temperature for 1 h, emptied, washed as before and 50µl of specific anti-canine affinity purified antibody conjugated with horseradish peroxidase, diluted in MT-PBS added and incubated for another hour. For the IgG assay, sheep anti-dog IgG (h+l) (Bethyl, USA) conjugate was used at a dilution of 1/6000, for the IgA assay, goat anti-dog IgA (h) (Bethyl, USA) at a dilution of 1/1000, and for the IgE assay rabbit anti-dog IgE (Iatrics, Tempe, Arizona, USA) at a dilution of 1/50. Plates were emptied, tapped dry and washed three times with MT-PBS-T and three times with reverse osmosis purified-deionised water and tapped dry. Fifty µl of 3, 3', 5, 5'-tetramethylbenzidine (TMB)-substrate solution (Kirkegaard and Perry Laboratories, Gaitherburg, USA, No. 50-76-00) was added to each well and developed for 30 minutes at room temperature. The reaction was stopped by adding 50µl per well 1M orthophosphoric acid. The absorbance value at 450nm (A450) was determined in a Dynatech MR 5000 plate reader (Dynatech, Guernsey, Channel Islands) with each plate blanked against the no antigen control well. The A450 value of each well was corrected to a pre-determined reference positive A450-value of 1.0 (IgG) or 0.5 (IgA and IgE) for a defined positive control serum (5/4), calculated by multiplying the read value with a correction factor. The correction factor was calculated by dividing the pre-determined reference A450 value by the mean of the test positive control A450 value (McLaren *et al.* 1981). Intra- and inter assay variations were within 10 to 15%. To minimise variations, all 908 sera were tested for each Ig class on the same day.

3.3.1. Standardisation of the ELISA

Cut-off values for the ELISA (to discriminate between positive and negative reactors) were determined for each antibody class by calculation from the mean + 4 standard deviations (sd) of a panel of 153 Australian dogs (Table 5). These included helminth-free dogs, dogs with monospecific infections of various helminths (*Taenia hydatigena*, *T. ovis* and *T. pisiformis*, *Ancylostoma caninum*, *Toxocara canis*, *Trichuris vulpis*, *Dipylidium caninum* or *Dirofilaria immitis*) and 88 dogs from a metropolitan area, known not to have been exposed to *E. granulosus*, but exposed to a range of other helminth and protozoan parasites (Johnston and Gasser 1993).

All positive sera were re-tested for all three specific antibody classes and positive results confirmed.

3.4. Abattoir data from sheep

Sheep slaughtered at the Stanley Butchery for human consumption were examined for evidence of hydatid cysts. Cyst material was taken to the laboratory at the Department of Agriculture and examined under a light microscope. If protoscolex material could not be detected microscopically, tissue samples were fixed in 10% formalin and submitted to the Auckland Animal Health Laboratory, Lynfield, New Zealand for histological examination. *E. granulosus* and *Taenia hydatigena* cysts were recorded.

Sheep slaughter data and recordings of *E. granulosus* and *T. hydatigena* cysts from home kills on farms in the Falkland Islands were collated from written returns, submitted by the farm owners to the Veterinary Office at regular intervals.

Differences between sheep data were statistically analysed using the chi-square test (Schwabe 1984).

4. Results

4.1. Abattoir data

In 1991, farm slaughter recorded a total of 15204 sheep slaughtered on farms in the Falkland Islands, of which harboured 434 *Cysticercus tenuicollis* (2.85%), the larval stage of *Taenia hydatigena* and 7 hydatid cysts, *E. cysticus* (0.05%). On West Falkland farms, a total of 4952 sheep (32.6% of the total numbers) had been slaughtered, with 138 (2.8%) sheep found to have cysts of *T. hydatigena*. Sheffield Farm, on West Falkland reported one *E. granulosus* cyst in its home kill in 41 sheep slaughtered (2.44%). On East Falkland farms, 10252 (67.4%) sheep were killed with a total of 296 (2.88%) animals with cysts of *T. hydatigena* and 6 (0.06%) with *E. granulosus* cysts. The Murrell Farm reported 3 cysts in a total recorded home kill of 99 sheep (3.03%), Smylie's Farm one case in 232 (0.43%), North Arm 1 in 1879 (0.05%) and Bleaker Island 1 in 72 (1.39%) (Table 2).

At the Stanley abattoir, a total of 4349 sheep were slaughtered during 1991, of which 159 (3.66%) had one or more cysts of *T. hydatigena* and 5 (0.11%) *E. granulosus* cysts present. 4253 (97.8%) of the sheep came from East Falkland farms. The *E. granulosus* cysts were found in sheep originating from five farms on East Falkland, each with one sheep harbouring a hydatid cyst. Goose Green supplied a total of 1637 sheep, thus the Goose Green specific annual hydatid incidence was 0.06%, Horseshoe Bay 107 (0.93%), Douglas 139 (0.72%), Fitzroy 370 (0.27%) and Salvador 759 (0.13%) (Table 2).

Farm slaughter records for 1992 show 16616 sheep slaughtered in the Falkland Islands, of which 4842 (29.1% of the total numbers) were slaughtered on West Falkland. There 173 (3.57%) sheep harboured cysts of *T. hydatigena*, but none a hydatid cyst. East Falkland reported 11774 sheep slaughtered on the farms, with 476 (4.04%) infected with cysts of *T. hydatigena* and one hydatid cyst (0.008%). This hydatid cyst was recorded from one of 1074 sheep killed at Mount Kent Farm (0.09%).The total number of infected sheep detected for the entire geographic region was 649 with cysts of *T. hydatigena* (3.91%) and 1 with a *E. granulosus* cyst (0.006%) (Table 3).

At the Stanley butchery, the total number of sheep slaughtered in 1992 was 6187, of which 357 (5.77%) had cysts of *T. hydatigena* and 18 (0.29%) hydatid cysts. The hydatid cysts were found in sheep originating from 10 different locations. Nine farms were located on East Falkland, which supplied a total of 5730 sheep for slaughter (92.6%). The specific annual incidence for the farms with hydatid cysts were as follows:

Goose Green 2 sheep (0.14%) with cysts in 1472 sheep supplied, Horseshoe Bay one sheep (0.7%) with a cyst in 143 sheep, Fitzroy one sheep with a cyst (0.09%) in 1162 sheep, Port Louis one sheep with a cyst (0.21%) in 473 sheep, Johnson's Harbour two animals with cysts (0.68%) in 295 sheep, King's Ridge 1 sheep (1.1%) with a cyst in 91 sheep, Salvador 4 sheep with cysts (1.02%) in 393 sheep, Motley Island 2 sheep with cysts (1.11%) in 180 sheep and Mount Kent 2 sheep with cysts (1.92%) in 104 sheep. West Falkland sheep constituted 7.4% of the total kill at the Stanley abattoir. Only one farm supplied sheep to the butchery from West Falkland, Keppel Island, and of those 2 sheep (0.44%) out of 457 harboured a hydatid cyst (Table 3).

Farm slaughter returns recorded a total of 12278 sheep slaughtered in the first 11 months of 1993 (ending 30. Nov. 93), with 555 (4.5%) sheep with cysts of *T. hydatigena* recorded, and a total of 5 sheep (0.04%) with cysts of *E. granulosus*. West Falkland farm returns accounted for 3278 (26.7%) of the total recorded number of sheep slaughtered on Falkland farms, with 131 (4.0%) sheep with cysts of *T. hydatigena* and two (0.06%) sheep with hydatid cyst. The two sheep with *E. granulosus* cysts were recorded at Port Howard in a total of 686 sheep slaughtered (0.14%) and at Pebble Island in 286 sheep (0.35%). East Falkland records cover 9000 (73.3%) sheep, with 424 (4.7%) sheep with cysts of *T. hydatigena* and 3 (0.04%) sheep with hydatid cysts. One was recorded in 595 sheep (0.16%) slaughtered at Johnson's Harbour, one other in one of 144 sheep (0.7%) from Bombilla farm, and one in 40 (2.5%) sheep on Bleaker Island (Table 4).

The inspection records for the first 11 months of 1993 show the total number of sheep slaughtered through the Stanley abattoir as 4832, with 366 (7.6%) sheep with cysts of *T. hydatigena* and 23 (0.48%) sheep harbouring *E. granulosus* cysts. One of the sheep with a hydatid cyst had been supplied from West Falkland, Pebble Island from a total of 562 sheep slaughtered (0.2%). East Falkland farms supplied 4270 (88.4%) sheep for slaughter, with 22 (0.51%) sheep harbouring hydatid cysts and 299 (7.0%) sheep cysts of *T. hydatigena*. Hydatid cysts were found in 2 of 313 sheep originating from Goose Green (0.6%), in 2 of 296 sheep from Elephant Beach (0.67%), in one of 430 from Fitzroy (0.23%), in 2 of 310 sheep from Johnson's Harbour (0.65%), in six of 351 sheep from Kingsford Valley (1.7%), in one of 477 from Moody Valley (0.2%), in 3 of 274 sheep from Cape Dolphin (1.1%), in 4 of 474 animals from Motley Island (0.8%) and in 1 out of 49 sheep from Long Island (2.0%) (Table 4).

Numbers of sheep with *E. granulosus* cysts found at the Stanley abattoir, for the last two years (1992 and part 1993) are significantly higher than those recorded on farms (χ^2 =

43.41 and 39.44, respectively; $p<0.01$), but not in 1991 ($\chi^2 = 2.829$; $p>0.05$). The recordings of sheep harbouring *E. granulosus* cysts at the Stanley abattoir have risen significantly from 1991 to 1993 ($\chi^2 = 9.89$; $p<0.01$), but not between 1991 and 1992 ($\chi^2 = 3.63$; $p>0.05$) and 1992 to 1993 ($\chi^2 = 2.469$; $p>0.05$).There is, however a significant rise in the number of sheep with *E. granulosus* cysts recorded in the Falkland Islands as a whole between 1991 and 1993 ($\chi^2 = 8.889$; $p<0.01$) and from 1992 to 1993 ($\chi^2 = 5.57$; $p<0.05$), but not from 1991 to 1992 ($\chi^2 = 0.69$; $p>0.05$). Sheep with hydatid cysts are also significantly more prevalent over the study period in East Falkland sheep compared with West Falkland sheep ($\chi^2 = 5.99$; $p<0.05$).

Numbers of sheep with cysts of *T. hydatigena* have risen significantly each year of this study (1991 to 1992: $\chi^2 = 54.93$; $p< 0.01$; 1992 to 1993: $\chi^2 = 20.03$; $p<0.01$) and are now at a comparable level with the levels recorded in the mid eighties (Whitley 1983, Table 12). Similar to the recordings of sheep harbouring hydatid cysts, prevalence figures at Stanley are significantly higher each year than they are suggested from Camp records of sheep slaughter for the same year (1991: $\chi^2= 13.74$; $p< 0.01$; 1992: $\chi^2 = 37.04$; $p< 0.01$; 1993: $\chi^2 = 63.39$, $p< 0.01$).

Table 2: Numbers of sheep slaughtered on farms and at the Stanley abattoir in 1991 and *T. hydatigena* (*C. tenuicollis*) and *E. granulosus* (*E. cysticus*) cysts recorded (percentages in brackets).

Location		Total numbers of sheep slaughtered	*E. granulosus* cysts	*T hydatigena* cysts
FARM HOME KILL				
West Falkland:				
	Total	4952 (32.6)	1 (0.02)	138 (2.8)
Sheffield		41	1 (2.44)	14 (34.1)
East Falkland:				
	Total	10252 (67.4)	6 (0.06)	296 (2.88)
Bleaker Island		72	1 (1.39)	0 (0)
Murrell Farm		99	3 (3.03)	0 (0)
North Arm		1879	1 (0.05)	0 (0)
Smylie's		232	1 (0.43)	2 (0.86)
Falklands	Total	15204 (100)	7 (0.05)	434 (2.85)
STANLEY ABATTOIR KILL				
West Falkland:				
	Total	96 (2.2)	0 (0)	3 (3.1)
East Falkland:				
	Total	4253 (97.8)	5 (0.11)	156 (3.66)
Goose Green		1637	1 (0.06)	89 (5.44)
Horseshoe Bay		107	1 (0.93)	2 (1.8)
Douglas Station		139	1 (0.72)	4 (2.8)
Fitzroy		370	1 (0.27)	6 (1.62)
Salvador		759	1 (0.13)	37 (4.87)
Falklands	Total	4349 (100)	5 (0.11)	159 (3.66)

Table 3: Numbers of sheep slaughtered on farms and at the Stanley abattoir in 1992 and *T. hydatigena* (*C. tenuicollis*) and *E. granulosus* (*E. cysticus*) cysts recorded (percentages in brackets)

Location		Total number of sheep slaughtered	*E. granulosus* cysts	*T. hydatigena* cysts
FARM HOME KILL				
West Falkland:				
	Total	4842 (29.1)	0 (0)	173 (3.57)
East Falkland:				
	Total	11774 (70.9)	1 (0.008)	476 (4.04)
Mount Kent		1074	1 (0.09)	7 (9.03)
Falklands	Total	16616 (100)	1 (0.006)	649 (3.91)
STANLEY ABATTOIR KILL				
West Falkland:				
	Total	457 (7.4)	2 (0.44)	15 (3.28)
Keppel Island		457	2 (0.44)	15 (3.28)
East Falkland:				
	Total	5730 (92.6)	16 (0.27)	343 (5.98)
Goose Green		1472	2 (0.14)	69 (4.69)
Horseshoe Bay		143	1 (0.7)	4 (2.8)
Fitzroy Farm		1162	1 (0.09)	64 (5.50)
Port Louis		473	1 (0.21)	44 (9.30)
Johnson's Harbour		295	2 (0.68)	28 (9.49)
King's Ridge		91	1 (1.1)	7 (1.09)
Salvador		393	4 (1.02)	35 (8.91)
Motley Island		180	2 (1.11)	13 (7.22)
Mount Kent		104	2 (1.92)	10 (9.62)
Falklands	Total	6187 (10)	18 (0.3)	357 (5.77)

Table 4: Numbers of sheep slaughtered on farms and at the Stanley abattoir in 1993 and *T. hydatigena* (*C. tenuicollis*) and *E. granulosus* (*E. cysticus)* cysts recorded (percentages in brackets).

Location		Total numbers of sheep slaughtered	*E. granulosus* cysts	*T. hydatigena* cysts
FARM HOME KILL				
West Falkland:				
	Total	3278 (26.7)	2 (0.06)	131 (4.0)
Port Howard		686	1 (0.14)	52 (7.6)
Pebble Island		286	1 (0.35)	4 (1.4)
East Falkland:				
	Total	9000 (73.3)	3 (0.03)	424 (4.7)
Johnson's Harbour		595	1 (0.16)	7 (1.2)
Bombilla		144	1 (0.7)	0 (0.0)
Bleaker Island		40	1 (2.5)	0 (0.00
Falklands	Total	12278 (100)	5 (0.04)	555 (4.5)
STANLEY ABATTOIR KILL				
West Falkland:				
	Total	562 (11.6)	1 (0.2)	67 (12.0)
Pebble Island		562	1 (0.2)	67 (12.0)
East Falkland:				
	Total	4270 (88.4)	22 (0.05)	299 (7.0)
Goose Green		313	2 (0.6)	19 (6.0)
Fitzroy		430	1 (0.23)	27 (6.27)
Johnson's Harbour		310	2 (0.65)	15 (4.83)
Elephant Beach		296	2 (0.67)	26 (8.78)
Long Island		49	1 (2.0)	1 (2.0)
Kingsford Valley		351	6 (1.7)	33 (9.4)
Moody Valley		477	1 (0.2)	34 (7.1)
Cape Dolphin		274	3 (1.1)	31 (11.3)
Motley Island		474	4 (0.8)	34 (7.2)
Falklands	Total	4832 (100)	23 (0.48)	366 (7.6)

4.2. ELISA

4.2.1. Standardisation of the ELISA

The cut-off value for the ELISA was determined using 153 sera from dogs, which had no known exposure to *E. granulosus.* The mean of 153 absorbance values at 450 nm (Appendix 3) + 4 standard deviations (sd) for all immunoglobulin classes (IgG, IgA and IgE) tested was used as the cut-off value. An absorbance value greater than the cut-off was regarded as a positive reaction. The respective values were 0.561 (IgG), 0.533 (IgA) and 0.270 for the IgE assay (Table 5).

The frequency distribution of absorbance values for the negative control group (suburban and rural dogs and helminth-free, mono-specifically infected dogs) relative to the respective cut-off absorbance value chosen, showed some absorbance values of negative sera exceeding the cut-off for a positive reaction in the ELISA. The test specificity was thus 99.3% for the IgG assay, with the absorbance value for one of the 153 sera exceeding this absorbance value, 98.7% for the IgA assay (two exceeding the cut-off limit) and 99.3% for the IgE assay (one value exceeding the chosen cut-off), respectively. The combined specificity for the test system was therefore calculated to be 97.4% (Figures 3, 4 and 5).

In comparison with the absorbance values of the negative control group (n=153), the Falkland Islands sera gave a high "background" in ELISA, especially in the IgG and IgE assay. The mean absorbance for the Falklands samples in the IgG assay was more than twice that of the negative control group, with the standard deviation (sd) also being almost twice as high as for the controls (Table 6). In the IgA assay, the mean was greater for the Falklands group, and the sd almost twice as large than that for the control group (Tables 5 and 6). The mean absorbance in the IgE assay for Falkland samples was three times greater than that of the negative control group, and the sd value was almost twice as high (Tables 5 and 6). Two groups within the Falkland Islands dog population assumed to be regularly (and reliably) dosed with praziquantel by the veterinary officer, were potential negative control groups. One group was the Stanley dog population and the other the Royal Airforce Police guard dogs at the military garrison at Mount Pleasant Airport (MPA). The police dogs at MPA are all of the German Shepherd Breed or it's crosses. Means and standard deviations of the absorbance values, were similar to those of the total Falkland dog population, and thus higher than for the control group of 153 Australian non-infected dogs (Tables 5 and 6). High "backgrounds" in ELISA can be the result of cross-reactivity with other helminth infections, or a consequence of diet and

breed (Gasser *et al*. 1988, 1989). With the cut-off absorbance values set using sera from the 153 Australian dogs, the Stanley dog population would have contained four positive reactors. Since these were very unlikely to have had exposure to *E. granulosus*, the reactions were presumed to be false positive reactions (Table 6, Appendix 4). It was assumed that the higher "background" reactivity in the Falkland Islands sera influenced the specificity of the test system, and resulted in the number of falsely positive test results.

In a group of 74 *E. granulosus* infected dogs from New South Wales, Australia, the sensitivity of the ELISA test system, ie. the ability to exclude false negative reactions and to determine whether a dog had been infected with *E. granulosus* was 75.6%, if just one absorbance value above the cut-off in any one of the three antibody classes was used to determine infection. This sensitivity was reduced to 58.1% when the criterion for a positive test specified that two absorbance value had to be above the cut-off to constitute a positive test result, and that one of the absorbance values for a positive serum had to be at least 0.1 above the cut-off (Appendix 5; Tables 7 and 8). While this decreased the test sensitivity, it increased the specificity of the test to 100%.

It was decided to follow-up with priority only those dogs which had one absorbance result 0.1 above the cut-off OD in one of the three assays, and an absorbance value above the cut-off in at least one of the other two assays. This would eliminate the false positive ELISA results, such as they were assumed in the Stanley group, but retain a degree of sensitivity (58.1%) that was superior to the sensitivity of arecoline hydrobromide purging, which can be as low as 10% (Wachira *et al.* 1990). Under these conditions, the test system would still be able to detect the majority of dogs with specific antibodies to *E. granulosus*.

Table 5: Mean of absorbance values at 450 nm and standard deviations (in brackets) of groups of negative and positive control sera from Australian dogs tested individually in ELISA for specific IgG, IgA and IgE antibodies against *Echinococcus granulosus.*

Group	No. of dogs tested	IgG	IgA	IgE
***Echinococcus granulosus* uninfected**				
Rural and suburban dogs (Victoria)	88	0.131 (0.107)	0.188 (0.095)	0.052 (0.053)
Experimental dogs	65	0.164 (0.097)	0.158 (0.080)	0.037 (0.059)
Total	153	0.145 (0.104)	0.173 (0.090)	0.046 (0.056)
Helminth free dogs (negative controls)				
	1	0.15	0.2	0.08
	1	0.080	0.1	0.05
***Echinococcus granulosus* infected**				
Natural infection (New South Wales)	74	0.749 (0.414)	0.583 (0.418)	0.333 (0.139)
Experimentally infected (positive control sera)				
	1	1.0	0.50	0.50
	1	0.80	0.40	0.35

The absorbance cut-off (co) value of the Ig-ELISA's was calculated as follows:

A450(co) for the IgG-ELISA = 0.145 + 4 x 0.104 = 0.561

A450(co) for the IgA-ELISA = 0.173 + 4 x 0.090 = 0.533

A450(co) for the IgE -ELISA = 0.046 + 4 x 0.056 = 0.270

Table 6: Mean of absorbance values and standard deviations (in brackets) of sera from Falkland Islands dogs, dogs from Stanley and the garrison at Mount Pleasant airport tested individually in ELISA for specific IgG, IgA and IgE antibodies against *Echinococcus granulosus.*

Group	No. of dogs tested	IgG	IgA	IgE
Police dogs*	17	0.316 (0.125)	0.091 (0.057)	0.080 (0.059)
Stanley	31	0.408 (0.156)	0.150 (0.088)	0.070 (0.056)
Falklands (Range)	908	0.361 (0.172) (0.001-1.199)	0.178 (0.164) (0.000-1.524)	0.151 (0.093) (0.000-1.088)

* Mount Pleasant Airport Royal Air Force Police guard dogs

Figure 3: Frequency distribution of IgG-ELISA absorbance values for *E. granulosus* uninfected dogs (n=153, black bars) and Falkland Islands dogs (n=908, shaded bars). A positive reaction is > 0.561.

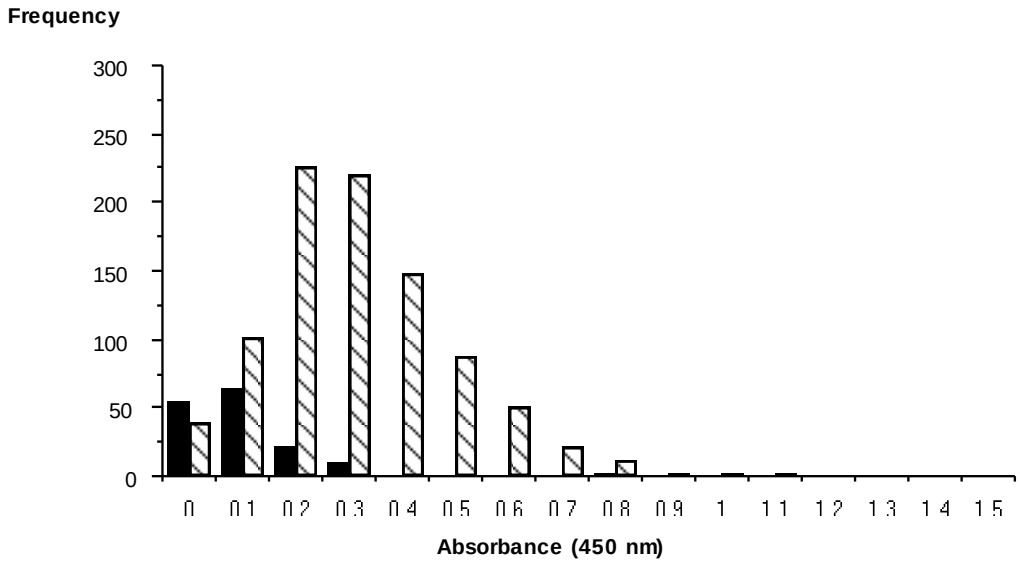


Figure 4: Frequency distribution of IgA-ELISA absorbance values for *E. granulosus* uninfected dogs (n=153, black bars) and Falkland Islands dogs (n=908, shaded bars). A positive reaction is > 0.533.

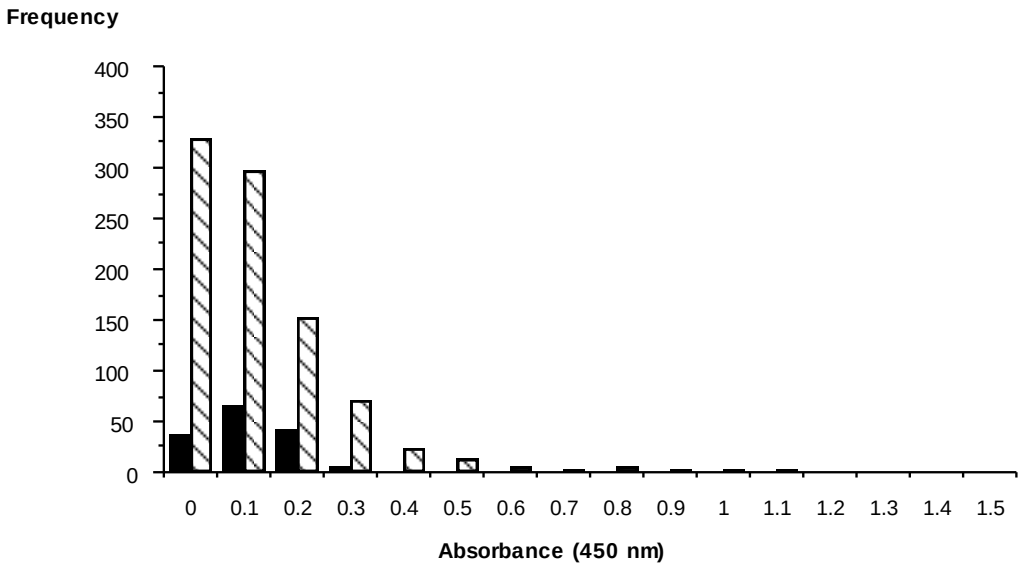


Figure 5: Frequency distribution of IgE-ELISA absorbance values for *E. granulosus* uninfected dogs (n=153, black bars) and Falkland Islands dogs (n=908, shaded bars). A positive reaction is > 0.270.

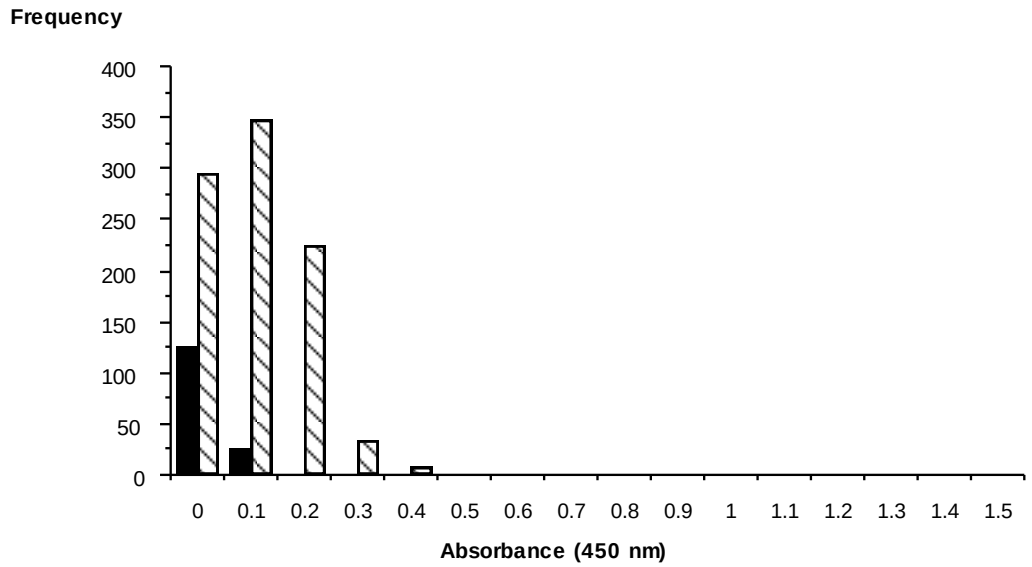


Table 7: Sensitivity and specificity of the serum antibody ELISA with the criterion for a positive result of a single absorbance value above the cut-off in either of three antibody classes (IgG, IgA or IgE) in a group of 74 naturally *E. granulosus* infected dogs from New South Wales, Australia and 153 naturally *E. granulosus* uninfected rural and suburban dogs from Victoria, Australia.

	E. granulosus infected	Not *E. granulosus* infected	
pos. Test result	56	4	60
neg. Test result	18	149	167
Total	74	153	227

Sensitivity = 56/74 = 75.6% Specificity = 149/153 = 97.4%

Table 8: Sensitivity and specificity of the serum antibody ELISA with the criterion for a positive result of two absorbance values above the cut-off in the three antibody classes (IgG, IgA and IgE) in a group of 74 naturally *E. granulosus* infected dogs from New South Wales, Australia and 153 naturally *E. granulosus* uninfected rural and suburban dogs from Victoria, Australia.

	E. granulosus infected	Not *E. granulosus* infected	
pos. Test result	43	0	43
neg. Test result	31	153	184
Total	74	153	227

Sensitivity 43/74 = 58.1% Specificity 153/153 = 100%

4.2.2. Anti-*E. granulosus* serum antibodies

Blood samples were obtained from 908 dogs over the age of six months and transported to Melbourne. These dogs came from 91 farms, Stanley and the military garrison at Mount Pleasant Airport (MPA). In Melbourne, the sera were tested in ELISA for specific anti-*E. granulosus* antibodies (IgG, IgA and IgE) (Appendix 6).

The mean absorbance value for all the Falkland sera was 0.361 in the IgG-ELISA (standard deviation (sd) 0.172), 0.178 (sd 0.164) for the IgA-ELISA and 0.151 (sd 0.093) for the IgE-ELISA (Table 6).

Sixteen dogs (1.76%), located in 9 distinct locations of the Falkland Islands, four on West Falkland and five on East Falkland were positive for anti-*E. granulosus* antibodies (Figure 6, Table 9). Eleven dogs from West Falkland locations were test-positive, with four dogs (out of 81) from three farms and one house in the Hill Cove settlement area. This resulted in a sero-prevalence of 5% for the Hill Cove settlement area. Five (out of 90) dogs in the wider Fox Bay settlement area had a positive test result (sero-prevalence 5.5%), and one dog each in South Harbour, out of 7 (14%) and Shallow Harbour, out of 7 (14%). There are no records of *E. granulosus* cysts in any sheep in these locations in the past three years (Figure 6, Table 9). Five dogs came from five East Falkland farms, and 3 dogs were located on farms which had *E. granulosus* cysts identified in their sheep in the previous three years. One test-positive dog out of 32 was located at Fitzroy (farm sero-prevalence 3.1%), one out of 14 at Port Louis (7.1%) and one out of 62 at North Arm (1.7%). Of the remaining dogs, one dog out of a group of 7 was located at Riverview Farm (14%) and one (out of 10) at Greenfield Farm (10%). There had been no records of hydatid cysts in sheep in either of these latter two farms (Figure 6, Table 9). At Greenfield farm however, the questionnaire revealed a history of *E. granulosus* cysts in sheep purchased from other farms, yet none of these cysts had previously been reported.

Table 9: Absorbance values (450 nm), locations and identity of 16 dogs with specific anti-*E. granulosus* IgG, IgA and IgE in the first test (1°), absorbance values at re-bleeding, absorbance values of 7 dogs positive at the second bleed (2°) and recordings of hydatid cysts in sheep in these locations 1991-1993.

Location		Dog ID.	Absorbance			Interval between bleeds (months)	Number of sheep with hydatid cysts
			IgG	IgA	IgE		
South Harbour	1°	48	0.359-	0.740+	0.381+	ND	0
Leicester Falls	1°	124	0.787+	0.603+	0.292+		0
	2°		0.979+	0.622+	0.264-	6	
Shallow Harbour	1°	144	0.680+	0.225-	0.294+	ND	0
Stoney Ridge	1°	304	0.706+	0.106-	0.277+		0
	2°		0.568+	0.266-	0,272+	6	
Port Edgar	1°	308	0.562+	1.430+	0.012-		0
	2°		0.310-	0.507-	0.156-	6	
Port Edgar	1°	313	0.735+	0.139-	0.300+		0
	2°		0.268-	0.340-	0.161-	6	
Fox Bay West	1°	329	0.565+	1.105+	0.245-		0
	2°		0.521-	0.282-	0.172-	6	
Westley Farm	1°	487	0.600+	0.333-	0.384+		0
	2°		0.626+	0.869+	0.105-	4	
Mossvale Farm	1°	507	1.096+	0.451-	0.280+		0
	2°		0.235-	0.396-	0.215-	4	
Hill Cove	1°	510	1.199+	0.963+	0.063-		0
	2°		0.840+	0.877+	0.149-	4	
West Lagoons	1°	518	0.833+	0.628+	0.060-		1
	2°		0.231-	0.355-	0.164-	4	
Riverview	1°	548	0.690+	1.524+	0.231-	Died	0
Fitzroy Farm	1°	628	0.896+	0.800+	0.251-		3
	2°		0.447-	0.597+	0.179-	3	
Port Louis	1°	640	1.010+	0.594+	0.354+		1
	2°		0.670+	0.385-	0.266-	3	
Greenfield Farm	1°	676	0.769+	0.298-	0.285+		0
	2°		0.599+	0.505-	0.120-	3	
North Arm	1°	875	0.721+	0.542+	0.184-	ND	1
New sero-positive							
Coast Ridge		41	0.903+	1.132+	0.190-	10	0
South Harbour		54	0.811+	1.096+	0.237-	10	0
Coast Ridge		190	0.682+	0.676+	0.156-	5	0
Westley Farm		488	0.672+	1.202+	0.031-	4	0
Westley Farm		489	0.675+	0.693+	0.195-	4	0
West Lagoons		517	0.736+	0.602+	0.192-	4	0
Greenfield		679	0.574+	0.981+	0.143-	3	0

ND = not done

Figure 6: Total number of dogs examined and number of sero-positive dogs, number of sheep examined and number of sheep with hydatid cysts in settlements identified by anti-*E. granulosus* ELISA (*T.h.= T. hydatigena, E.g.=E. granulosus*)

Location	Number of dogs		Number of sheep		
	examined	positive	examined	positive	
				T.h	*E.g*
West Falkland					
Fox Bay Area	90	5	4422	195	0
Hill Cove	81	4	696	0	0
Shallow Harbour	7	1	732	7	0
South Harbour	7	1	294	10	0
East Falkland					
Fitzroy	32	1	2438	126	3
Riverview	7	1	30	0	0
Port Louis	14	1	1436	145	1
North Arm	62	1	5006	216	1
Greenfield	10	1	107	1	0

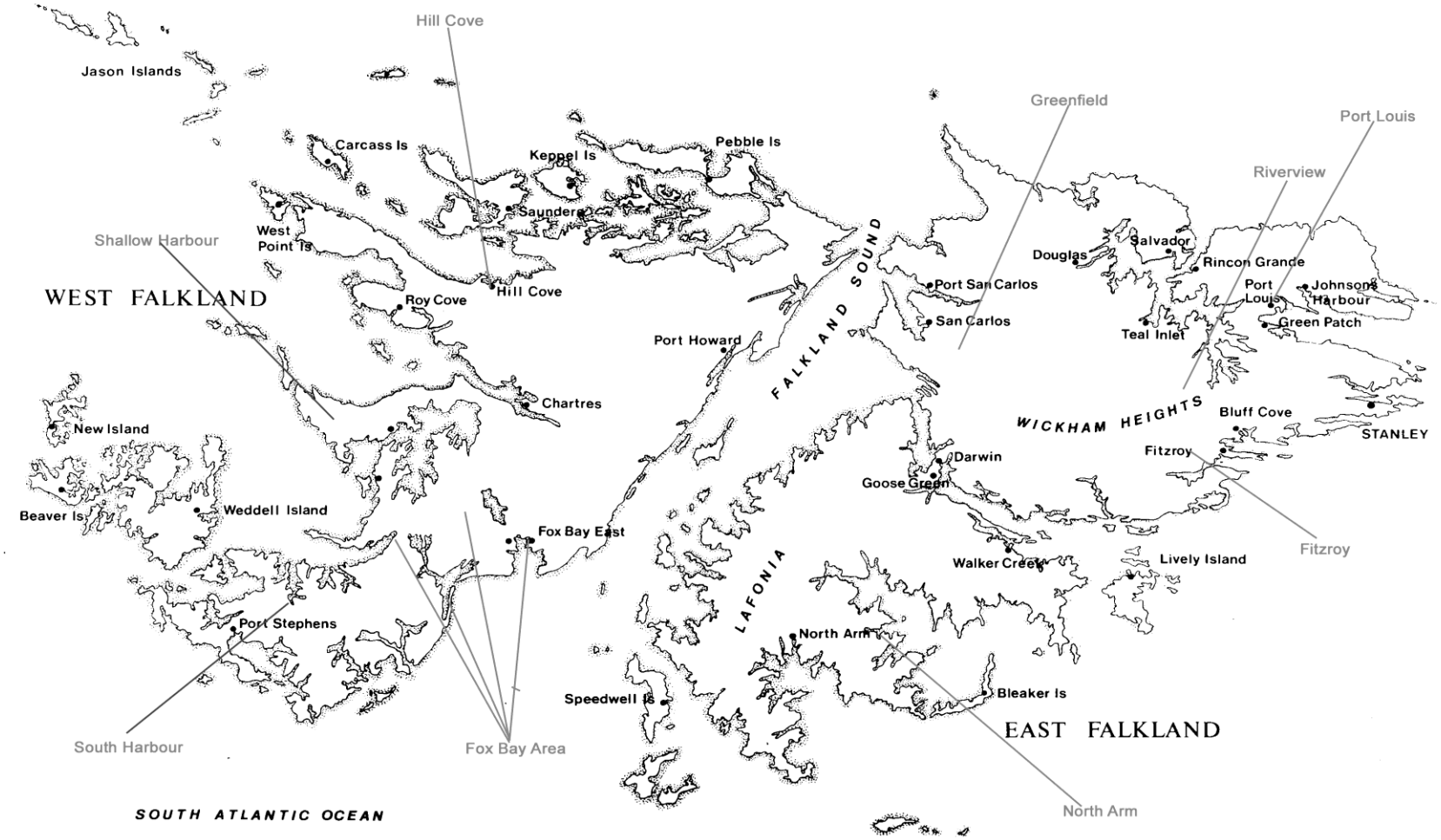


Figure 6: Location of sero-positive dogs and concurrent findings of hydatid cysts in sheep on farms in the Falkland Islands

4.2.3. ELISA results of re-bleeds

Serum samples were obtained from 73 dogs and tested by ELISA in Melbourne. Twelve sera were from dogs which had been identified as test-positive by the first ELISA assay. Of these twelve sera, only three were now test-positive when tested the second time, one from a dog (No. 124) in the Fox Bay area, at Leicester Falls farm, six months after it had been bled the first time and another two located at Hill Cove (Nos. 487 and 510). For these latter two dogs the interval between first and second bleeding was four months. The other nine sera had absorbance values which had fallen below the cut-off value in the three Ig assays in five of the cases, with the other four not fulfilling the criterion of two positive assays to be considered test-positive. The interval between bleeds for these dogs ranged from 3 to 6 months (Appendix 7) (Table 9).

Out of the other 61 sera, seven dogs showed a test-positive reaction. All but two of these were located on farms which had already been identified in the first assay. These two dogs were located on one farm (Coast Ridge), however, which lies in the Fox Bay area. The interval between first and second bleeding for all 61 dogs ranged from 3 to ten months (Table 9).

4.3. Arecoline hydrobromide purging

Of the 16 dogs with positive test values in the serum antibody ELISA, 12 (75%) were purged with arecoline hydrobromide.

None of the 12 purges revealed adult *E. granulosus* or their proglottides. Eggs of *Toxocara* spp. and hookworms (*Ancylostoma caninum* or *Uncinaria stenocephala*) were found in 50%, and 100% of the purges, respectively. No adult *Toxocara*, hookworms or *T. hydatigena* were found in the purges.

4.4. Questionnaire

Questionnaires were answered by 29 dog owners in 3 settlements and 20 farms, all within the areas identified by test-positive results in the first ELISA. Thirteen were owners of dogs which had been identified as positive reactors in the ELISA. One owners dog, which had previously had a positive test, was not available for re-testing.

All dogs were fed a diet that consisted predominantly of raw sheep meat (mutton), with most dog owners (16/29; 55.2%) slaughtering the sheep themselves. Only six dog owners (20.7%) considered the sheep killing facility on their property, or from where they obtained the mutton for dog food, as dog-proof, ie. denying dogs access to sheep body fluids or offal. One of those facilities had only been upgraded to dog-proof status within the last year.

21 (72.4%) of the dog owners questioned had no recent knowledge of *E. granulosus* cysts, ie. not seen one within the last five years rising to 92.4% for owners of positive dogs. All owners had seen cysts of *T. hydatigena*. Twenty-four (82.8%) of dog owners dosed their own dogs with praziquantel (100% of owners of positive reactors) at the prescribed dosing intervals (6 weeks), five dog owners had the drug administered by the appointed dog inspector.

Offal disposal through burying of sheep livers and lungs was carried out by one (3.4%) of the dog owners (none of the positive dog owners), through burning by 9 (31%) owners (and 23% of positive dog owners), disposal into the sea by five owners (17.2%), but 30.7% of owners with positive dogs. Storage for a minimum of a month in a container (usually 44 gallon drum) was carried out by 11 out of 29 (37.9%) owners/farmers, and 53.8% of positive dog owners (Table 10).

Table 10: Answers to a questionaire (Appendix 1) presented to 29 dog owners, representing 73 dogs on East and West Falkland

Question		
Source of dog food:	Mutton (sheep) Beef (cattle) Geese	(29/29) (10/29) (11/29)
	Positive answer (%)	
	All dog owners (n=29)	Owners (n=13) of a dog found to be positive for anti-*E. granulosus* antibodies
Slaughter facility dog-proof?	20.7	15.4
Slaughtering themselves?	55.2	46.2
E. granulosus cysts in the last five years?	27.6	7.6
T. hydatigena cysts in the last five years?	100	100
Dose their own dogs with praziquantel?	82.8	100
<u>Offal disposal</u>		
by burial	3.4	0.0
by burning	31	23
by storage	37.9	53.8
into the sea	17.2	30.7

5. Discussion

5.1. Prevalence of E. granulosus in sheep

The offal inspection results from Stanley and the farms record *E. granulosus* and *T. hydatigena* cysts in sheep in the Falkland Islands in the period from 1991 to 1993. The total number of sheep with *E. granulosus* cysts recorded, ie. Stanley and Camp together, was 12 (0.06%) in 1991, with 5 sheep with *E. granulosus* being recorded at Stanley. In 1992, 19 (0.08%) sheep with *E. granulosus* cysts were recorded in the Islands as a whole, with 18 in just over a quarter of the total recorded in Stanley. The preliminary data for 1993 show 28 (0.16%) sheep with hydatid cysts for the entire Falkland Islands, yet 23 sheep were recorded at the Stanley abattoir where only just over a quarter of the total number of sheep slaughtered in the Islands was inspected. Inspections of sheep livers and lungs at Stanley tend to record significantly more hydatid cysts (1992 and 1993) than do inspections of sheep on the same farms by lay staff, ie. usually the farmers themselves.

The prevalence of sheep with hydatid cysts recorded at the Stanley abattoir rose significantly ($\chi^2 = 9.89$, $p<0.01$) from 1991, the first year of the study to 1993, the final year. And while the prevalence in 1993 is still lower than was shown by Whitley (1983) in his final year report for 1983 (Figure 2), it is significantly higher in 1992 ($\chi^2 = 5.18$, $p<0.05$) and the first eleven months of 1993 ($\chi^2 = 9.63$, $p<0.01$) than the lowest prevalence recorded in the Falklands in 1985 (Table 11, records of the Veterinary Office, Stanley).

More hydatid cysts are recorded from East Falkland sheep than they are from West Falkland sheep. This can be expected, as the Stanley abattoir procures it's sheep for slaughter almost exclusively from East Falkland farms (generally over 90% of the sheep) in the study period. This biases the sample towards East Falkland sheep. In general, the slaughter records suggest that, with the majority (53) of 59 hydatid cysts recorded between 1991 and 1993 on East Falkland farms, a residual *E. granulosus* problem is more prominent on the East of the Falkland Islands. Significantly higher numbers of hydatid cysts are recorded during inspection at the Stanley abattoir (by professionally trained staff) for sheep which originate from farms where farm slaughter records suggest a much lower prevalence. This is an indication of the problems lay staff on farms experience in the identification of hydatid cysts and the true prevalence of *E. granulosus* cyst for East Falkland, at least, is probably more closely reflected by the prevalence detected at the Stanley abattoir inspection, than by the farm inspection records.

Most sheep slaughtered at the Stanley abattoir are 7 to 8 years of age and are culled for their age from the farm flock. However some younger sheep are occasionally slaughtered, because of undesirable traits such as black pigmentation. One hydatid cyst was found in one such young two-years old ewe from Goose Green (Table 3) in 1992.

It had been predicted (Whitley 1983) that hydatidosis would be eradicated from the Falkland Islands by 1989. The records of sheep offal inspection on farms and, the more reliable, inspection results from Stanley, suggest that transmission has still been taking place as late as 1990, especially with the evidence of a hydatid cyst in a two-years-old sheep. With egg production per adult worm ranging from 100 to 1500 per proglottis (Arundel 1972, Rausch 1975, Thompson and Eckert 1982), it is likely that more than one sheep was infected at Goose Green. The remainder of that sheep's age cohort are not likely to be slaughtered or killed, if present farm practises continue, until 1997 when they will be seven to eight years old. *E. granulosus* cysts in sheep remain viable for the remainder of the intermediate host's life (Gemmell *et al.* 1986). Therefore, a potential for transmission exists until 1997 and longer if members of the age cohort survive beyond then. Hydatid eradication efforts will thus have to be maintained for at least another 5 or six years.

If the 1992/93 prevalence data are extrapolated over the whole Islands sheep population of over 700000 (anon. 1993), approximately 3500 Falkland sheep could be expected to be infected with *E. granulosus*. This presents considerable opportunities for infection. While the distribution of infected sheep may not be uniform over the whole of the Islands, clusters of *E. granulosus* infected sheep will occur in certain locations, e.g. Goose Green and other farms where hydatid cysts continue to be found, presenting a higher risk of infection there than in other areas (Figure 6, Tables 2 to 4).

The low number of hydatid cysts recorded for sheep on West Falkland suggests that the *E. granulosus* eradication campaign has been more effective there than on East Falkland, where cysts appear more numerous. As no or few sheep from West Falkland have been slaughtered through the Stanley abattoir, due to the difficulties and costs of transporting to Stanley, the recording of hydatid cysts on West Falkland is done by farmers. The problems untrained farmers experience when inspecting sheep on East Falkland are probably shared by West Falkland farmers, and thus the true prevalence on West Falkland may be higher than is suggested by home kill records. Until professional inspection of West Falkland sheep can be achieved, the true prevalence of *E. granulosus* on West Falkland remains unknown.

Inspection records for the Stanley abattoir and the farms show a large number of *T. hydatigena* cysts in Falkland Islands sheep. There is variation between farm inspection records and those from the Stanley abattoir, and the figures are generally significantly higher at Stanley than they are on East and West Falkland farms.

The overall (Stanley and Camp) prevalence of cysts of *T. hydatigena* for 1991 is 3%, 4.4% for 1992 and 5.38% for part of 1993, and shows a rising trend. Figures at the Stanley are consistently higher (3.66%, 5.77%, 7.6% and rising significantly) than the numbers recorded on farms in the same periods, and suggest that the true prevalence of *T. hydatigena* approaches the levels of prevalence of the mid eighties (Table 11). Records from farms are incomplete and obtained by lay staff and as with *E.* granulosus records it can be assumed that records of cysts of *T. hydatigena* obtained at Stanley more accurately reflect the true prevalence of this parasite in the Falkland Islands. The rise in the annual incidence and the level obtained suggest that eradication efforts are not controlling the parasite any longer.

The high and rising prevalence of *T. hydatigena* does not present a public health problem in itself, as the presence of *E. granulosus* does. It provides evidence, however, that *T. hydatigena* successfully completes its life cycle and therefore suggests that *E. granulosus* transmission may also be possible because the life cycles of the two parasites are similar. The adult stage of *T. hydatigena* in the dog is treated successfully with praziquantel with a similar degree of efficacy as is observed in the treatment of *E. granulosus*. The pre-patent period of *T. hydatigena* is also comparable, if somewhat longer than for *E. granulosus* (34 to 58 days), being 52 to 66 days (Deplazes and Eckert 1988a). The six-weekly praziquantel treatment which is being administered in the Falkland Islands could therefore be expected to terminate infection with *T. hydatigena* in the dog before the adult worm matures and produces eggs.
The fecundity of *T. hydatigena* is much greater than that of *E. granulosus*, with the production of eggs per proglottis reported to be as high as 2429 to 7622 eggs (Deplazes and Eckert 1988b), which increases the biotic potential of *T. hydatigena*. The larva (*C. tenuicollis*) can be found attached to the serosal surfaces of the abdominal cavity, as well as the liver and intestines, and can also be found freely in the abdominal cavity itself. Freshly killed, untreated mutton is the common diet of Falklands sheep dogs (see results of the questionnaire, p. 63). Viable cysts could be fed to dogs without breach of the current Falkland Islands legislation. Control programmes in other countries have found it more difficult to restrict the life cycle of *T. hydatigena* than the one of *E. granulosus* (Gemmell 1958). The high numbers of the larval stage of *T. hydatigena* in the Falkland

Table 11: Number of sheep inspected at the Stanley abattoir and on farms (Camp), prevalence of cysts of *Echinococcus granulosus* and *Taenia hydatigena* in the Falkland Islands from 1984 to 1990 (Records of the Veterinary Office, Stanley) (Percentages in brackets).

	Stanley			Camp		
Year	Number of sheep slaughtered	*T. hydatigena*	*E. granulosus*	Number of sheep slaughtered	*T. hydatigena*	*E. granulosus*
1984	6704	547 (8.2)	29 (0.4)	13429	nd	233 (1.74)
1985	2519	163 (6.5)	1 (0.04)	15912	nd	233 (1.5)
1986	4451	188 (4.2)	4 (0.08)	16817	nd	134 (0.8)
1987	nd	nd	nd	15122	nd	30 (0.2)
1988	nd	nd	nd	15974	nd	30 (0.2)
1989	nd	nd	nd	15327	nd	48 (0.3)
1990	nd	nd	nd	16682	nd	43 (0.26)

nd = no data

Islands, which has been applying praziquantel regularly for nearly fifteen years suggests deficiencies in the application of the drug. Only if praziquantel was not, or only irregularly, ie. after an interval considerably longer than the prescribed 42 days applied, would the adult *T. hydatigena* worm be able to proceed to patency and produce eggs.. Under these circumstances, the potential for the transmission and completion of the life cycle of *E. granulosus* still exists, with a number of sheep in the Falkland Islands, possibly as high as 3500 (if the prevalence data in found in sheep in this study are extrapolated over the entire national flock) still currently infected with the larval stage of *E. granulosus*.

5.2. Standardisation of the ELISA

Diagnostic tests such as ELISAs require the determination of cut-off values that distinguish between uninfected individuals and infected ones within a population. Choosing the cut-off value too low may result in a number of uninfected individuals to be falsely diagnosed as infected and the specificity (number of test-negative, uninfected individuals over total number of uninfected individuals, Table 12) of the test system will decline. A cut-off value chosen too high will result in the test system failing to detect a number of truly infected individuals in the population, and the sensitivity of the test system (number of infected, test-positive individuals over total number of infected individuals, Table 12) is affected. The cost of a misdiagnosis, ie. whether the test system identifies individuals either falsely as test-positive or falsely as test-negative may vary, depending on the situation in which the test system is used (Vizard *et al*. 1990). Misdiagnosing an individual as either infected or uninfected must be measured against the number of individuals which are correctly identified by the test system.

The performance of the test within a population can be expressed as the predictive value (positive and negative), which is a measure of the cost of misdiagnoses. The positive predictive value describes the number of truly infected, test-positive individuals over the total test-positive individuals, while the negative predictive value is calculated from the number of truly uninfected, test-negative over the total test-negative individuals. Predictive values are dependent upon the sensitivity and specificity of the test and the prevalence of the condition that is being detected in the population. As the prevalence of the condition declines, the positive predictive value (PPV) decreases as well (assuming sensitivity and specificity of the test remain the same), ie. the ratio of truly infected individuals against non-infected within the test-positive number of individuals decreases (Table 12, Figure 7). From available historic prevalence data on hydatid cysts in sheep, a

Table 12: Sensitivity, Specificity and predictive value of a diagnostic test system

	Diseased	Not diseased	
Test positive	a	b	a+b
Test negative	c	d	c+d
	a+c	b+d	Total

Sensitivity = a/a+c

Specificity = d/b+d

Positive predictive value = a/a+b

Negative predictive value = d/c+d

Figure 7a: Predictive values of a test with 75% sensitivity, 95% specificity at increasing prevalence of the condition detected (● positive predictive value, □ negative predictive value)

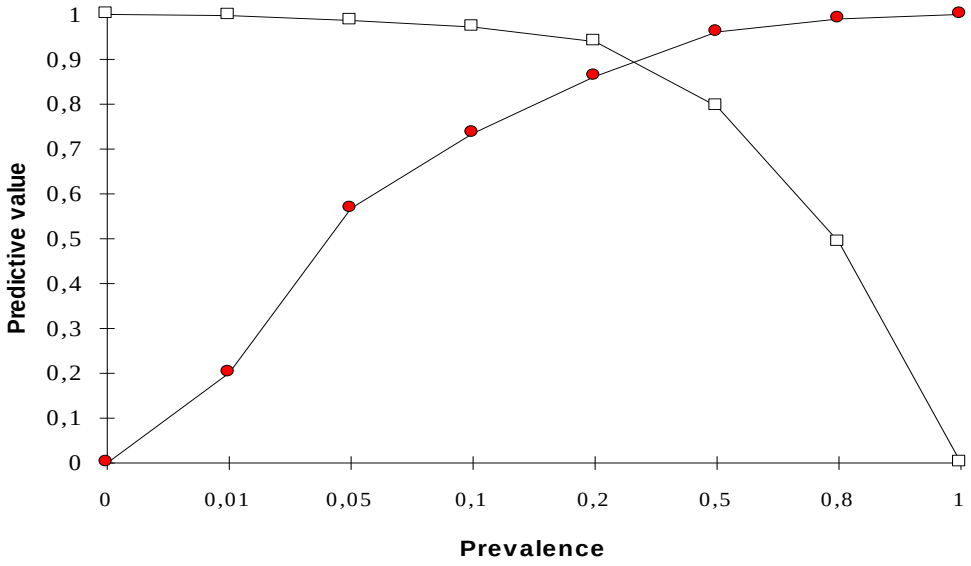
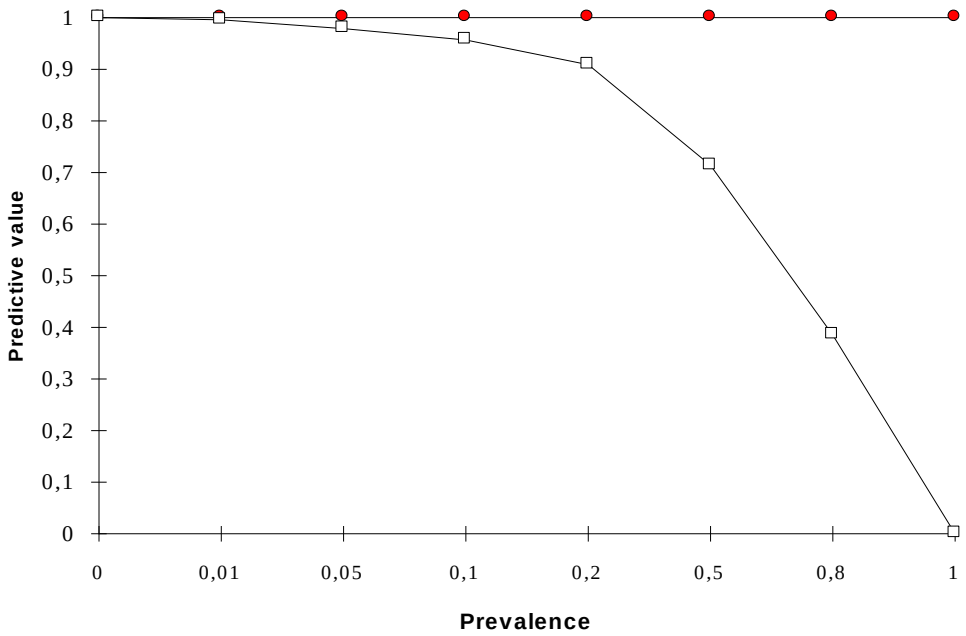


Figure 7b: Predictive values of a test with 60% sensitivity, 100% specificity at increasing prevalence of the condition detected (● positive predictive value, □ negative predictive value)



low prevalence in sheep in the Falkland Islands was predicted and it was reasonable to expect a low sero-prevalence in the dog population. Figure 7 shows the theoretical PPV's for the ELISA with two different combinations of sensitivity and specificity, the first for a discriminating criterion of one absorbance value above the cut-off (Figure 7a), the other with the discriminating criterion for a positive test requiring two absorbance values to be greater than the cut-off value (Figure 7b).

If a test system with a low positive predictive value was to be used in this study, the large number of false positive (uninfected/unexposed dogs) would distort the epidemiological picture and make the test unsuitable for an assessment of the current status of echinococcosis in the Falkland Islands. The number of test-positive dogs could not be distinguished reliably from those which were truly infected/exposed or not infected/not-exposed. With the low assumed prevalence, a test with a high sensitivity, but more importantly also high specificity was required in order that the positive predictive value of the test be high enough to allow a valid assessment of echinococcosis in the Islands.

Cut-off values for serological tests, which ultimately determine sensitivity and specificity have been defined in various ways. Some workers have defined the cut-off value as the mean absorbance plus two (Coker-Vann *et al*. 1984) or three standard deviations (Gottstein 1984) of a group of uninfected control individuals, arbitrarily (Gasser *et al.* 1993) or as the absorbance value which is higher than the highest absorbance value of any uninfected, negative control individual (Heath *et al*. 1985). Negative and positive control group for the standardisation of the ELISA would ideally be recruited from the same geographic region.

There were no known infected dogs in the Islands which could have served as positive controls. Viable cyst material could have been obtained from the Stanley abattoir and used for experimental infections however, the lack of high security quarantine facilities meant that a proposal to infect Falkland dogs experimentally with *E. granulosus* could not be carried out.

Two potential negative control groups existed within the Falkland Islands: a group of 17 military guard dogs at the garrison at Mount Pleasant Airport and a population of dogs in Stanley. Both groups were regularly treated with praziquantel under the supervision of the veterinary officer and in the case of the military dogs fed only a commercial diet, with no access to offal. However, the latter group consisted exclusively of German Shepherd dogs (and crosses) and had all come from the United Kingdom within the last

one to five years, while the Falkland Islands dogs were almost exclusively Border Collie types, and crosses thereof. The Stanley dogs comprised several breeds and crosses, including retired farm dogs, mainly Border Collies, a few German Shepherds and other breeds. After analysis of the absorbance values of these two "indigenous" negative control groups, the high "background" reactivity in the ELISA's made them unsuitable as negative controls. High background absorbance values can be the result of variations in the immune status, due to factors such as diet, breed, age and genetic constitution (Gasser *et al*. 1988, 1989). Additionally, some exposure to *E. granulosus*, while unlikely, could not be completely excluded. The resultant high cut-off values would not allow to discriminate between truly non exposed/infected dogs and low reacting exposed/infected ones, resulting in a low test sensitivity.

To reliably establish sensitivity and specificity of the test, control sera from Australian dogs were used in this study. These sera were from dogs found to be infected with *E. granulosus* (n=74) and experimental and rural and city dogs not known to have been exposed to or infected with *E. granulosus* (n=153). The mean absorbance value for the uninfected dogs plus four standard deviations was chosen as the diagnostic cut-off value and resulted in high specificities in all three assays, with a combined specificity for the test of 97.3%, and sensitivity of 74.3%. The theoretical positive predictive value of the ELISA, assuming a sero-prevalence of 1%, was calculated to be 20%, ie. for every true positive the test would identify four dogs falsely as positives (Figure 7a). The chosen cut-off values, however, identified four Stanley dogs (assumed to be unexposed/infected) as test-positive. With an additional requirement that the absorbance values of two antibody classes should be above the cut-off value (with one 0.1 above), the ELISA did not identify any individual Stanley dog (falsely) as positive. This increased the test specificity to 100% and resulted in a sensitivity of 58.1%, as observed in the group of sera derived from 74 Australian dogs demonstrated to have been infected with *E. granulosus*. This test sensitivity was lower than could have been achieved with the criterion which required that the absorbance value of a single antibody class be above the cut-off value. It was much higher, however than could be expected from arecoline hydrobromide purging, which can have a sensitivity as low as 10% (Wachira *et al*. 1990). The theoretical PPV increased from 20% to 100%, minimising the number of false-positives in the group of test-positive dogs (Figure 7b).

After standardisation of the ELISA, one could be confident that the assay would identify only dogs which had developed specific antibodies against *E. granulosus* and were highly likely to have been exposed to/infected with *E. granulosus*. With a sensitivity of 58.1% the test system would fail to identify a proportion of dogs which had been

exposed/infected, however, the numbers of dogs which would be identified should allow an assessment of the epidemiological situation of *E. granulosus* in the Falkland Islands and identify geographic regions where there had been exposure.

5.3. Prevalence of anti-E. granulosus serum antibodies in dogs

Sixteen (1.76%) of 908 dogs in the Falkland Islands were found to be sero-positive for anti-*E. granulosus* antibodies. The sero-positive dogs were found in nine distinct Falkland locations, five on East and four on West Falkland, with a high farm-specific sero-prevalence. The highest total number of test-positive dogs was in the Fox Bay area and the Hill Cove settlement area on West Falkland and Port Louis and Riverview farm on East Falkland (Figure 6, Table 9). In the Fox Bay area, two dogs were sero-positive at Port Edgar farm alone, and at Hill Cove all four sero-positive dogs, while belonging to different farms/owners, all still live in the settlement.

On East Falkland, the detection of sero-positive dogs in the settlement at Fitzroy farm, at North Arm and in Port Louis coincides with the recording of hydatid cysts in sheep slaughtered at the Stanley abattoir, or, in the case of North Arm on the farm itself.

The detection of specific antibodies in dogs from West Falkland suggests that dogs are still being exposed to *E. granulosus*. This is in contrast to the low recordings of *E. granulosus* cysts in sheep, either from the infrequent numbers slaughtered through the Stanley abattoir, or from records supplied from farm kills. There had been no reports of hydatid cysts in sheep originating from Hill Cove in the three years of this study. The clustering of sero-positive dogs at Hill Cove, owned by different owners may suggest a common source of exposure to *E. granulosus* cysts. Sheep and cattle offal disposal facilities need to be investigated for deficiencies which may allow dogs access to ruminant livers and lungs infected with *E. granulosus* cysts (see below).

The identification of four dogs in the wider Fox Bay area, with two at Port Edgar, one at Stoney Ridge farm and one at Fox Bay West and Leicester Falls farm indicate recent exposure to *E. granulosus* material in these locations. No sheep from these properties had been reported to be infected with hydatid cysts in the three years of this study, neither at the Stanley abattoir or in sheep slaughtered on the farm.

Also the identification of single sero-positive dogs at Shallow and South Harbour does not match with the recording of hydatid cyst in sheep from the same farm. The presence

of specific antibodies suggests, however, exposure to *E. granulosus* parasite material on these farms which should be investigated.

The presence of sero-positive dogs on West Falkland suggests that exposure/infection of dogs and hence transmission of *E. granulosus* to the intermediate host may have occurred recently in these locations. Sheep may present a risk of infection to dogs at these locations for some time to come, and efforts should be made to prevent the repeated infection of dogs with *E. granulosus*. Thus, safe offal disposal and praziquantel treatment need to be maintained.

The detection of dogs with specific antibodies against *E. granulosus* on East Falkland at Fitzroy, Port Louis and North Arm coincides with the recordings of hydatid cysts in sheep from those farms. In most cases, hydatid cysts in sheep may be the result of transmission a number of years ago, but the presence of specific antibodies in these East Falkland dogs suggests that the conditions which have led to transmission may still exist now. While the exact length of time for which *E. granulosus* specific antibodies are detectable in dogs is not known (Gasser *et al*. 1993) other studies on canine taeniasis suggest that they may persist for several weeks, even after the removal of worms by anthelmintic treatment (Jenkins and Rickard 1985, Heath *et al*. 1988). The presence of specific anti-*E. granulosus* antibodies in a dog at Riverview identify another farm, where exposure to *E. granulosus* has happened recently, however, there were no records of hydatid cysts in sheep from this property within the study period.

The sero-positive dog at North Arm had been lost at a sheep gather and not returned to the farm for three months. It was bled immediately after it's return, and the presence of specific antibodies suggests that it had had exposure to and may have been infected with *E. granulosus*.

The serological screening of dogs for specific anti-*E. granulosus* antibodies has confirmed that dogs are exposed to *E. granulosus* material in some Falkland locations. The presence of sero-positive dogs on West Falkland farms suggests that *E. granulosus* material has been accessible to dogs on these farms, despite the apparent absence indicated by the lack of recordings of hydatid cysts in sheep from these properties for a number of years. Hydatid cysts may have been present and were missed during inspection on the farm, or have been wrongly identified as cysts of *T. hydatigena* or *Corynebacterium pseudotuberculosis* lesions. Training of farmers to correctly identify hydatid cysts is necessary to obtain accurate records of hydatid cysts in sheep.

5.4. Arecoline purging

Arecoline hydrobromide purging did not demonstrate *E. granulosus* in any of the dogs that had been identified to have specific anti-*E. granulosus* antibodies. This result is to be expected for several reasons. Because of the long period of time that it took to collect all blood samples from the Falkland Islands' dog population, and then transfer these samples to Melbourne for testing there was necessarily an interval of varying length between the collection of the first blood sample and the arecoline purge (and second bleed). This interval varied from 3 to 6 months. All dogs would have been subjected to several and at least one mandatory praziquantel treatment since the bleed. It is likely that praziquantel treatments during this period would have eliminated any pre-patent or patent *E. granulosus* infections in those dogs. Thus they could not be detected upon purgitation. Praziquantel is also readily available to all dog owners and stocked on all farms, thus the possibility exists that dog owners treated their dogs in anticipation of the visit. While efforts were made to reduce the period of fore-warning of the impending veterinary visit, some dog owners may still have had sufficient time to treat their dogs with the cesticidal drug prior to the arecoline purge.

Arecoline purgation has been shown to have a sensitivity as low as 10%, and thus may fail to demonstrate *E. granulosus* in a large proportion of infected dogs (Wachira *et al*. 1990). This is substantiated by the results of the 12 purges in this study. Eggs of other worms (*Toxocara* spp. and hookworms) were found upon faecal flotation, yet the purges failed to detect any helminth, including *T. hydatigena*.

The ELISA results of the second blood sample taken from 12 of the 16 dogs identified as sero-positive in the first assay, show a decline of the absorbance values for the majority of dogs. 10 dogs of the original 12 have absorbance values in the second assay below the criterion of positivity suggesting a decline of antibody levels. This is consistent with exposure to *E. granulosus* antigen which has been terminated several weeks ago and a subsequent decline in serological response, as has been reported in other canine taeniasis (Jenkins and Rickard 1985, Heath *et al*. 1988). Three dogs still fulfil the criterion for a positive reaction, one at Leicester Falls, and the other two at Hill Cove. The repeat ELISAs from other dogs in Hill Cove reveal a further three sero-positive dogs, on properties which had already been identified in the first assay. This suggests that the exposure to *E. granulosus* is still continuing.

This continued exposure appears to occur at South Harbour as well, and also on Greenfield farm on East Falkland. The deficiencies in the operation of the hydatid

eradication effort on these farms need to be investigated with priority, and exposure to *E. granulosus* in these locations stopped.

The detection of two additional sero-positive dog at Coast Ridge farm identifies this particular farm for the first time. Coast Ridge farm, however, is situated in the Fox Bay area and the test thus identifies dogs with *E. granulosus* exposure in the Fox Bay area, approximately 5 to 10 months after the first bleed. This suggests that the conditions leading to exposure to *E. granulosus* also still persist in the Fox Bay area.

5.5. Questionnaire

The questionnaire results of 29 dog owners indicate several areas, where the present hydatid eradication effort may be failing to prevent transmission of *E. granulosus* completely.

Only 20% of dog owners, and only 15% of dog owners with sero-positive dogs consider their killing facility (including offal disposal) for sheep and cattle to be "dog-proof", and several examples of "non-dog-proof" slaughter facilities can be found on properties which have been identified as having sero-positive dogs (Plates 1 to 3). Dog-proofing of the slaughter facilities has been a cornerstone of the present legislation (anon. 1981) and required the dog-proofing of slaughter facilities which did not comply within 12 months of the Hydatids (Dogs) Eradication Ordinance coming into force. It is therefore surprising that so many properties have not complied with this part of the legislation.

Many of the newer farms have been the result of sub-division within the last ten years, and new farmers have established their settlements in new locations on their property, or on previously abandoned sites. The dog-proofing of slaughtering facilities may have been the last priority in their effort to establish their new farms. They do not appear to have been aware of the existing legislation and the requirements in regards to slaughter facilities, and these, therefore need to be reinforced.

Only 27% of all dog owners and 7% of owners of sero-positive dogs had seen a hydatid cyst in sheep in the five years prior to being asked. While this may be a reflection of the declining prevalence of hydatid cysts in sheep over those years, it may reflect that these owners, many of which (at least 46%) are also slaughtering sheep, may not be able to recognise hydatid cyst during slaughtering. The prevalence of hydatid cysts on their properties would be perceived to be nil, resulting in a false sense of security and

possibly complacency about hydatid eradication rules. This danger exists particularly with owners of sero-positive dogs, who are 4 times less likely to recognise a hydatid cyst, because they have not seen one for years. They lack the ability to identify them accurately and this may also explain the absence of any report of a finding of hydatid cysts in sheep from areas, particularly on West Falkland, which had dogs identified as sero-positive.

Educational campaigns have to be directed at training all farmers to identify *E. granulosus* cysts, particularly those slaughtering their own sheep. These efforts have to be repeated frequently in order that the ability to identify hydatid cysts accurately is retained by the farming community. It is only then that one can be confident that sheep slaughter returns from farms will reflect the true prevalence of hydatid cysts in farm slaughter returns. None of the dog owners deliberately allowed their dogs access to ruminant offal, but offal disposal on a farm may open avenues for the transmission of *E. granulosus* material to dogs. Out of the four means of disposal, throwing the offal into the sea showed a distinct discrepancy between dog owners with sero-positive dogs and the questioned owners as a whole group. While 30% of owners with positive dogs used this method of disposing of ruminant offal (over the end of the wharf), only 17% of all dog owners used it. It is immediately obvious that offal which is being disposed of into the sea may be washed up again soon after on the shore and thus become accessible to dogs, the disposal generally also attracts a sizeable number of scavenging birds. Birds have been implicated as vectors in the transmission of *T. hydatigena* (Torgerson *et al*. 1992) and may have some significance in the transmission of hydatid infection, although this is rather unlikely. This means of offal disposal also contravenes the present legislation, which requires the drainage of slaughter facilities not only to be dog, but also bird-proof (Appendix 1). It is also noticeable that this form of offal disposal is the method of preference in the Hill Cove settlement which has a strong cluster of sero-positive dogs. While this relationship is only circumstantial, and no *E. granulosus* eggs or other worm material been demonstrated in dogs at Hill Cove, other methods of offal disposal, such as burning or burying may have an inherently greater capacity of preventing access of dogs to potentially infectious material.

6. Conclusions

In the past twenty years, the Falkland Islands hydatid eradication campaign has been largely based on three cornerstones: (1) the denial of access to offal for dogs, (2) the six-weekly administration of a cesticidal drug (praziquantel) and (3) dog control. Strict adherence to these measures have meant that the campaign in the Falkland Islands has largely followed the pattern of other successful campaigns, namely the ones in New Zealand and Tasmania. As in the latter two countries, the campaign in the Falkland Islands was initially mainly community based, with professional people, such as veterinary surgeons not permanently based in the Islands until 1976. Transmission of *E. granulosus* to humans ceased early on in the Falkland Islands, as perpetuation of the life cycle of the parasite became less frequent.

The prevalence of hydatid cysts in sheep started to fall soon after the first control measures had been taken and reached a low in 1985 of 0.04%. Data collected in this study put current prevalence in sheep slaughtered through the abattoir at Stanley, where more reliable data are obtained, between 0.11 to 0.48%, rising significantly towards 1993. This significant rise in prevalence in sheep since 1985 is cause for concern. Of even greater concern must be that transmission appears to have occurred as late as 1990 or 1991. This is substantiated by the fact that a cyst was found at the Stanley abattoir in a two-years-old sheep from Goose Green during the study in 1992. Eradication efforts will have to be continued until at least 1997 or until the age-cohort of the *E. granulosus* infected sheep at Goose Green detected in 1992 has been culled from the national flock, whichever is the later.

The serological testing of Falkland Islands dogs has identified 16 (1.76%) of 908 dogs tested with specific anti-*E. granulosus* antibodies. While this number may appear high, given the long-running campaign with six-weekly praziquantel dosing, it is in line with expectations based on the number of hydatid cysts detected in sheep during this study. The ELISA has identified individual dogs with antibodies, yet the results should be seen as focussing on sheep properties where exposure of dogs to *E. granulosus* appears to be still occurring. The repeat bleeds, after an interval ranging from 3 to 10 months, of twelve of those sero-positive dogs, and 61 dogs located on the same properties, detected seven additional dogs with anti-*E. granulosus* antibodies All newly identified dogs, however, were located in the same geographical regions that had already been identified in the first test. Three dogs, identified in the first test as sero-positive, were positive also in the second testing. This would suggest that exposure to *E. granulosus* has not ceased on these farms during the course of this study. A total of 310 dogs are located on these

farms (a third of the Falkland Islands dog population) and may therefore, potentially still be at risk of becoming exposed/infected on those properties where the test has shown exposure to occur. Sheep inspection records from the Stanley abattoir concur with the present serological results on three farms on East Falkland, and provide further evidence that not only exposure of dogs to *E. granulosus* material occurred on these farms, resulting in the formation of specific antibodies, but patent infection in dogs must have been present as well at one time, leading to transmission of the parasite to sheep.

Efforts have to be made to diminish the potential for further exposure and infection of dogs. Initially, these should be focussed on the deficiencies in the implementation of the existing Falkland Islands legislation, the 1981 Hydatid Eradication (Dogs) Order.

The slaughtering of sheep creates the possibility of dogs gaining access to sheep offal and body tissues containing *E. granulosus* larval stages. Prevention of access of dogs to cystic material must be seen as the most critical measure in any hydatid campaign (Thompson *et al*. 1993). In the Falkland Islands, existing sheep slaughtering facilities need to be examined on all farms, with priority on those which have dogs positive for anti-*E. granulosus* antibodies. Dogs must be denied access to these slaughtering facilities at all times. If dogs are denied access to *E. granulosus* material, the life cycle of the parasite cannot be perpetuated. "Dog-proofing" of slaughter facilities has been part of the Falkland Islands legislation since 1981, and all slaughter facilities should have complied with the rules within twelve months. However, a large majority of farmers in the present study did not regard their slaughter facility as sufficiently dog-proof (questionnaire results). This suggests that non-compliance is widespread and is consistent with evidence obtained from a limited number of farms (Plates 1-3). It is possible that the slaughtering facilities on these farms were the focus of exposure to *E. granulosus*. These deficiencies should be addressed with urgency.

The disposal of ruminant offal into the sea at Hill Cove indicates another possible route for transmission of *E. granulosus*. This could occur directly through the offal being washed up again on the shore or perhaps indirectly through birds which have been implicated in the transmission of other taeniids (Torgerson *et al*. 1992). This practise may also contravene the present legislation, which aims to deny birds access to the drainage of a slaughter facility.

The current legislation (anon. 1981) stipulates the regular administration of praziquantel under the supervision of a government-appointed dog inspector. These were usually appointed to a large settlement in Camp and supervised the dosing of large numbers of

Plate 1: Sheep slaughtering facility at Leicester Falls farm, Fox Bay area

Plate 2: Sheep slaughtering facility at Hill Cove

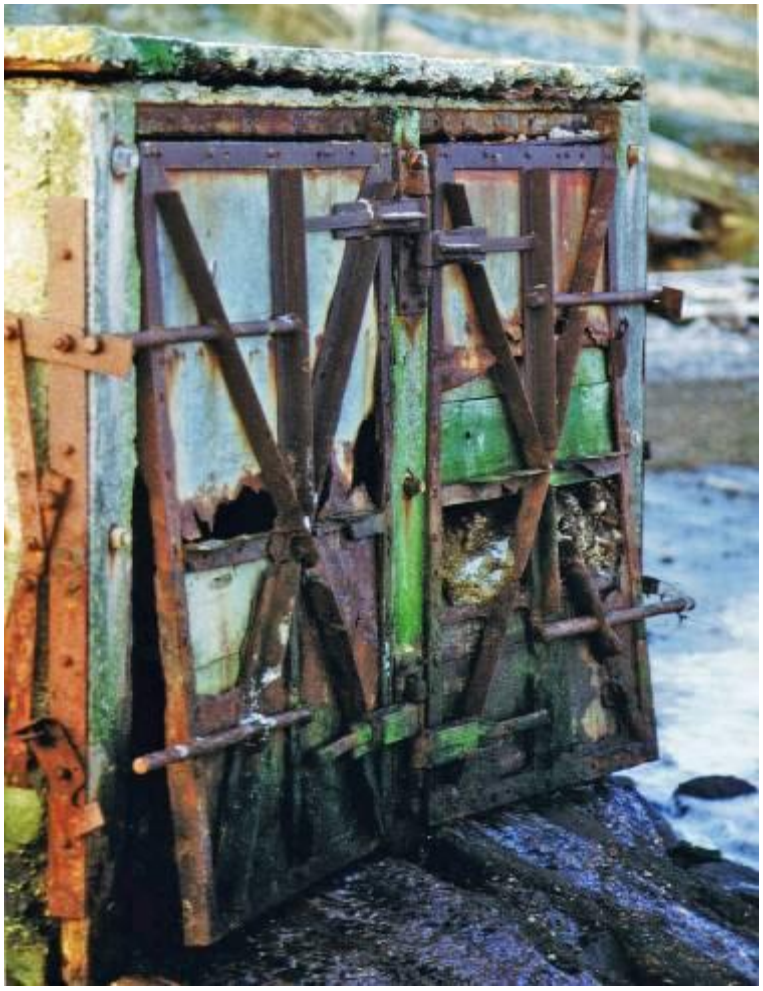


Plate 3: Offal storage at Fitzroy farm

dogs within that settlement. This is no longer the case as with the policy of sub-division and the development of over 90 smaller farms, dog-inspectors were no longer appointed. Most Falkland Islands dog owners (apart from Stanley and MPA) now dose their own dogs every six weeks (effectively being their own inspectors). The lack of supervision of the administration of praziquantel through an official may have allowed some dosing intervals to have become irregular or been missed altogether. The high prevalence of *T. hydatigena* does suggest that praziquantel treatments are being missed. In the larger settlements, including some which have shown sero-positive dogs (such as Hill Cove, the Fox Bay area, Fitzroy and North Arm) it is feasible and would be preferable, to re-introduce government-appointed inspectors to supervise the regular praziquantel dosing and adherence to the other stipulations of the present legislation.

The evaluation of the present campaign has been based on the collection of sheep data from farms around the Islands, as well as from the Stanley abattoir. Where data could be compared, mainly for some East Falkland farms, the prevalence of the larval stages of *T. hydatigena* and *E. granulosus* was significantly higher in sheep inspected at the Stanley abattoir than in sheep inspected on the farm. The majority of dog owners, who slaughter their own sheep, had not seen *E. granulosus* cysts for a number of years (questionnaire results), and the accuracy of their recordings has to be doubted. A significant number of farms does not submit returns on findings of hydatid cysts to the Veterinary Office. Others do not record cysts of *T. hydatigena* or *Corynebacterium pseudotuberculosis* abscesses found in their sheep in many of the slaughter returns to the Veterinary Office. Given the high prevalence of these pathogens in the Falkland Islands as a whole (*personal observations*) the accuracy of these data must be doubted. Sheep slaughter records suggested that *E. granulosus* had a much higher prevalence on East Falkland than on West Falkland, yet ELISA test results suggest more dogs have exposure to *E. granulosus* on West Falkland, than on East Falkland farms. While the true prevalence of hydatid cysts in sheep on West Falkland may not be known until a greater number of sheep is inspected by professionally trained meat inspectors, it is reasonable to assume that the prevalence on West Falkland is at least as high as it is in the East. The significant rise in the prevalence of hydatid cysts in sheep has only become apparent through the comprehensive inspection of all sheep offal at the Stanley abattoir. In conjunction with serological data, abattoir surveillance continues to identify farms where a risk of exposure of dogs to *E. granulosus* may be present. It is thus a useful tool not only in the monitoring of the progress of the eradication campaign but also for the identification of foci of residual *E. granulosus* infection. An additional benefit is also the educational value of serology, which may be used to bring ignorant or recalcitrant dog owners to compliance with the pertinent legislation (Thompson *et al*. 1993).

Opportunities for inspections of larger numbers of sheep offal exist from time to time, when large numbers of old-aged sheep are culled from the farm flock. Efforts need to be made to have at least some inspected by the veterinary officer, or under veterinary supervision, to gain a more accurate estimation of the prevalence of *E. granulosus* in sheep. Live sheep, mainly from West Falkland, are exported to Chile at regular intervals. In Chile, these sheep are slaughtered through an abattoir under veterinary supervision and the meat inspection records obtained from Chile would provide useful data which would help in the estimation of the prevalence of hydatid cysts in sheep. An export meat plant which is being planned for Stanley is another potential source of valuable data which may provide information on the future trends in prevalence.

Serological surveys for *Echinococcus* have shown a correlation between sero-prevalence and true parasitological prevalence in previous studies. However the ability of a serological ELISA for the determination of the infection status of an individual dog is sometimes unreliable (Gottstein *et al*. 1991, Deplazes *et al*. 1992). In this study an attempt was made to not only obtain serological evidence of the presence/absence of *E. granulosus* but also parasitological evidence of the parasite in the faecal matter from dogs. The diagnosis of current infection of dogs with *E. granulosus* relies on the demonstration of tapeworms, their segments or antigen in dog faeces. Arecoline purging and copro-antigen detection are possible alternatives. Both however, may have low sensitivity, especially in dogs with low worm burdens. Such dogs may escape detection (Wachira *et al*. 1990, Deplazes *et al*. 1992) and could be wrongly classified as not infected. Arecoline purging is known for its adverse side-effects on dogs and has been discredited in the Falklands. In 1977, prior to the introduction of praziquantel when *E. granulosus* had a much higher prevalence in the Falkland Islands (approx. 6 to 7% *E. granulosus*, 9 to 14% for *T. hydatigena*) 30 (75%) of 39 dogs purged successfully, however none of these purges revealed any *E. granulosus* and only two infection with *T. hydatigena* (Whitley 1983). None of the dogs purged in this study, previously identified as sero-positive, showed any *E. granulosus* in the arecoline purge. This result could be expected for several reasons: the interval that separated the two consecutive bleeds and the processing of the samples in ELISA in Melbourne was necessarily long (3 to 10 months) during which infected dogs may have spontaneously eliminated the worm from their intestines. Praziquantel dosing also continued to be used in the intervening period and dogs which may have been infected at the time of blood collection probably were not infected any more at the time of the purge. Arecoline purging has a low sensitivity (Gemmell 1973, Wachira *et al.* 1990) and may thus have failed to demonstrate *E. granulosus* in the purges of dogs even if they were infected.

A copro-antigen assay would be the preferred choice of diagnostic test for the detection of individual infected dogs. The combined application of this technique and serology has been shown to identify over 95% of dogs with *E. granulosus* infection (R. B. Gasser, *pers. comm.*). With likely foci of *E. granulosus* already identified by serology and through the detection of *E. granulosus* cysts in their sheep, any future copro-antigen detection survey should focus, in the first instance, on the farms identified in this study. Exposure to *E. granulosus* material is demonstrated by the specific antibodies detected in this study on those farms, and copro-antigen detection would be likely to be successful on those farms first.

Serological and copro-antigen assay surveillance should continue in the Falkland Islands on a regular basis, at least annually, in an attempt to identify the farms and locations where dogs are exposed to *E. granulosus.*

The number of dogs in the Falkland Islands, particularly on some of the larger settlements appears to be disproportionately high, and unnecessary for the nature and amount of work that is required from dogs. As the dog is the only definitive host in the Falkland Islands involved in the transmission of *E. granulosus,* the risk of transmission of the parasite is related to the number of dogs present. The large proportion (more than 90%) of dogs in the Islands live on farms, are regularly fed on raw mutton, and have frequent contact with sheep. Other countries, notably Cyprus, made their hydatid eradication campaign a success by reducing the number of dogs, either through de-sexing or the destroying of stray dogs. This reduced the overall dog population five-fold (anon. 1986, Polydorou 1976). Although there are no stray dogs in the Islands, a reduction of the number of dogs in the Falkland Islands to a minimal level (aided by the advent of contract sheep gathering gangs which utilise mechanised transport) should help eradicate *E. granulosus*. The number of dogs tested in this survey (908) is similar to the number of dogs present in the Islands in 1970. Whitley (1983) recorded about 800 to 860 dogs in the Falkland Islands between 1976 to 1983, down from 980 in 1970.

Disincentives to dog ownership could take the form of mandatory dog registration on farms, as is already practised in Stanley, with financial penalties if a certain number of dogs per farm/owner is exceeded. If, for example, the mean number of dogs per farm was reduced to 4 for the smaller farmlets, and 20 for the larger ones (a number which would still be sufficient for most requirements on the farm) the total number of dogs in the Falkland Islands would be halved. This would reduce the number of potential definitive hosts and thus further minimise the risk of hydatid transmission.

The positive serological reaction in the dog from North Arm, which had been stray for only a few months, suggests that the potential for exposure to *E. granulosus* is still present in the field. This suggests that any dog that strays from the farm, or is lost at a gather of sheep may be exposed to *E. granulosus* under such circumstances and therefore, should be treated with praziquantel as a matter of course upon its return to the owner and not left until the next regular praziquantel treatment is scheduled.

Control of hydatidosis/echinococcosis in the Falkland Islands rests on the denial of access to cyst material, yet the current legislation (anon. 1981) only prescribes that liver and lungs of ruminant animals need to be disposed of safely. Hydatid cysts have been known to occur in other sites than liver and lungs, including the kidneys and musculature (Shamsul Islam 1979), and other countries have prohibited the feeding of any ruminant material to dogs, unless it has been treated by heat or freezing (anon. 1986, anon. 1988). Similar restrictions on the feeding of raw ruminant flesh should be made in the Falkland Islands.

The origin of *E. granulosus* infection in the Falkland Islands are unclear, and no attempts have been made to identify its origins. The emergence of the first recorded hydatid cysts suggest the introduction towards the mid to end 1930's (Gibbs 1946), but both dogs or sheep may have been the carriers. Introductions of dogs would have been mainly from the United Kingdom, sheep carrying *E. granulosus* however could have originated from the United Kingdom, Argentina, Chile, Uruguay, New Zealand or Tasmania (Whitley 1983). The latter country would be of particular interest as the distinct Tasmanian strain of *E. granulosus* has a shorter pre-patent period (35 days) than the common mainland Australian sheep-dog strain (42 days) (Kumaratilake *et al*. 1983), and would thus be able to mature and produce eggs within the current prescribed praziquantel treatment interval. While it is impossible to carry out experimental infection studies in the Falkland Islands due to the absence of adequate facilities, efforts to identify the strain(s) of *E. granulosus* present in the Falkland Islands should be made (Lymbery and Thompson 1988), as the identity of strain may have direct and important implications for the dosing interval. If the strain of *E. granulosus* present in the Falkland Islands was of a Tasmanian origin, the present six-weekly dosing regime would need to be shortened to monthly treatments.

The introduction of sheep or dog from outside into the Falkland Islands bears the risk of re-importation of *E. granulosus*. Historically dogs and sheep have been introduced from a number of countries (UK, Australia, New Zealand) which all continue to be endemic for *E. granulosus* infection (with possibly the exception of New Zealand). The treatment

of dogs is simple and all dog imports into the Falkland Islands are regularly treated with 5mg/kg bodyweight of praziquantel before they leave the country of origin and again prior to disembarking at Port Stanley (*pers. observation*).

Diagnosis and treatment of sheep is not currently possible and the importation of live sheep from countries, such as recently from Tasmania (Reichel 1992), where *E. granulosus*-infection of sheep is still recorded brings with it the risk of re-introduction of *E. granulosus*. In future, such risk could be minimised through the employment of artificial breeding techniques, e.g. embryo transfer, which allow the transfer of superior genetic material to the Falkland Islands without the risk of re-introduction of *E. granulosus*.

In summary, the conclusions of this study are:

1. Echinococcosis/hydatidosis has not been eradicated from the Falkland Islands to date and it appears to be exist at low prevalence throughout the entire geographic region. The annual recorded prevalence in sheep is rising and the recent finding of hydatid cysts in young sheep suggest that eradication will not be complete for at least a further 5 to 10 years. The increase in prevalence of cysts of *T. hydatigena* suggests deficiencies in the regular application of praziquantel, which may also create possibilities for the transmission of *E. granulosus*. These deficiencies need to be addressed with urgency.

2. Foci of possible recent exposure/infection of dogs to *E. granulosus* and risk factors which may have led to this exposure have been identified in the study. Deficiencies in the application of the current legislation (ie. non-dog-proof sheep slaughter facilities) need to be addressed with priority, and an educational effort maintained, highlighting the life cycle of *E. granulosus* and the main factors preventing its perpetuation.

3. Further surveillance needs to combine the use of serological studies with copro-antigen assays to screen the dog population for exposure/infection to/with *E.* granulosus and attempt to identify foci of current transmission of *E. granulosus.*

4. Future importations of sheep should be sourced from regions free of *E. granulosus* infection, or be restricted to embryo material only.

5. Further studies need to be aimed at the identification of the biological "strain(s)" of *E. granulosus* present in the Falkland Islands. Praziquantel dosing intervals may need to be adjusted in accordance with the pre patent period of strain.

7. Recommendations

1. The increasing prevalence of hydatid cysts in sheep between 1991 and 1993 has only been detected through comprehensive abattoir surveillance at Stanley. These data provide currently the most accurate indication of the dynamics of *E. granulosus* infection in sheep and must be continued. Efforts must be made to obtain more accurate records on prevalence in sheep on West Falkland, through veterinary examination of mass slaughters.

2. The serological survey of the Falkland Islands' dog population has identified properties where dogs are highly likely to be exposed to *E. granulosus*. Further investigations have identified potential risk factors which may have led to exposure. Repeat examinations have shown that exposure continues on these farms. Serological surveillance needs to continue on those properties (maybe at cost to the owners) to monitor the success of measures which have been taken to prevent further exposure to *E. granulosus*. Farms that are identified through abattoir surveillance to have infected sheep, should be monitored by regular serological assays of their dogs to assure that transmission to dogs does not occur.

3. Copro-antigen assays (Allan *et al*. 1992, Deplazes *et al*. 1992) should be instituted to detect individual dogs with current *E. granulosus* infection. This would complement the assessment obtained by serological tools (with a combined sensitivity of 95%) and allow, if desired, the prosecution of owners of infected dogs through the courts.

4. Deficiencies in compliance with the present hydatid legislation need to be addressed with urgency. On all farms, but with particular emphasis on those farms which have been identified in this study as allowing exposure of dogs to *E. granulosus,* sheep (and cattle) slaughter facilities need to be made "dog-proof". The disposal of offal (livers and lungs) and other ruminant tissues should be by burial or burning only. The system of dog inspectors, responsible for the administration of the legislation in the out-lying settlements, should be re-introduced, where it is practicable.

5. Questionnaire results have indicated a lack of knowledge about hydatidosis/echinococcosis in the farming community, especially as far as recognition of hydatid cysts in sheep and the life cycle of the parasite is concerned. An educational campaign should be mounted by the Department of Agriculture in conjunction with the Medical and Education Department. This should raise the level of awareness of hydatid disease, focus on the recognition of *E. granulosus* lesions in sheep and prevention of

transmission between the two hosts. To aid and monitor progress in this direction, it is suggested that the Hydatids Advisory Committee be re-established with members of the above departments, and include members of the farming community and public at large.

6. The present survey has identified several farms with sero-positive dogs in the Falkland Islands. These farms account for more than a third of the Islands' dog population. The prevalence of hydatid cysts recorded in sheep has risen significantly since 1985 and during the course of this study from 1991 to 1993, and there are indications that cysts will be found in the Falkland Islands for at least another five years, possibly even longer. At this point in time there can be no suggestion of relaxing the comprehensive and compulsory six-weekly dosing regime with praziquantel. Should future investigations suggest a Tasmanian origin for *E. granulosus* in the Falkland Islands, an even shorter treatment cycle (probably monthly) may need to be implemented.

7. Abattoir surveillance has shown that *E. granulosus* infection of sheep has occurred as late as 1990 or 1991. The prevalence of antibodies in dogs suggests that dogs were exposed to the parasite during the present study. Since the prevalence of infection is increasing in sheep, it is possible that accidental human infection may also have been occurred. A comprehensive serological survey of the human population was last carried out in 1988-89. The present study would suggest that screening focussed on the farms identified with sero-positive dogs, and through the finding of hydatid cyst in sheep would be warranted.

8. To safeguard the achievements of the hydatid eradication campaign in the Falkland Islands thus far, and prevent the re-introduction of the parasite from outside the Islands, the control of the importation of dogs, ie. the appropriate treatment with praziquantel on arrival in the colony needs to be maintained. Importations of sheep should either be sourced from regions where *E. granulosus* infection is not known to occur, require the quarantining of the imports if they arrive from *E. granulosus* endemic regions or should be in the form of embryos.

8. Summary

Echinococcus granulosus has been recorded in the Falkland Islands since 1941, and prevalence data in sheep reached over 50% in the early 1950´s. Human cystic hydatid disease was also recorded in the Falkland Islands, and an eradication campaign has been mounted since the early 1970´s. This eradication effort led to a rapid decline of *E. granulosus* cysts in sheep and apparent cessation of transmission to humans, however more recent data suggested a possible increase in prevalence.

An evaluation of the epidemiological status of echinococcosis/hydatidosis was carried out in the Falkland Islands between 1991 and 1993 using recently developed serological tools to identify dogs with anti-*E. granulosus* antibodies and infected sheep through abattoir surveillance.

Abattoir surveillance of sheep at the Stanley abattoir and on farms in the Islands showed a significant increase during the study in the prevalence of cysts of *E. granulosus* towards 1993. Hydatid cysts were more frequently found at the Stanley abattoir than on farms, and more often in sheep originating from East Falkland farms, than recorded in sheep from West Falkland. The prevalence of *E. granulosus* cysts in sheep slaughtered at Stanley increased from 0.11% in 1991, over 0.29% in 1992 to 0.48% in 1993. On farms the prevalence of *E. granulosus* cysts in sheep was 0.05% in 1991, 0.006% during 1992 and 0.04% during 1993. In 1992 one cyst of *E. granulosus* was recorded in a two-years-old sheep from Goose Green, indicating that transmission of the parasite occurred as recently as 1990.

Testing of sera of the entire dog population of this geographic region (n=908), over six months of age revealed 16 dogs (1.76%) with specific anti-*E. granulosus* antibodies, suggesting recent infection with or exposure to the parasite. Twelve of these dogs could be re-bled, after an interval of between three to ten months and purged with arecoline hydrobromide. None of the purges revealed any patent infection with adult *E. granulosus*, however, the low sensitivity of arecoline testing did not allow convincing evidence that infection was absent. A second bleed of these twelve sero-positive dogs and a further 61 dogs located on the same farms revealed an additional 7 sero-positive dogs, suggesting continuing exposure to/infection with *E. granulosus* in these locations.

Recordings of hydatid cysts in sheep were made on three out of five farms on East Falkland which also had sero-positive dogs. None of the farms on West Falkland which

had dogs identified as sero-positive had *E. granulosus* cysts recorded in any of their sheep during the study period.

Sero-positive dogs were confined to 9 distinct locations on East and West Falkland, with several farms having more than one sero-positive dog, and investigations on those farms revealed deficiencies in the disposal of lungs and livers of sheep, which may potentially allow dogs access to hydatid cyst material. Dogs also are fed untreated sheep meat (i.e. not frozen or cooked) and the treatment of all ruminant tissue which is being fed to dogs is strongly recommended. Disposal of lungs and livers into the sea, as it is practised on one farm, was identified as one potentially critical deficiency that may have led to a clustering of sero-positive dogs in this location.

This study suggests that echinococcosis is still present, and increasing in the Falkland Islands. From this study recommendations are being made regarding the future course of the Falkland Islands hydatid eradication campaign, suggesting the use of additional immuno-diagnostic tools (copro-antigen assay) to be used in conjunction with sero-epidemiology, to detect current /recent infection/exposure to *E. granulosus*. Continued and expanded abattoir surveillance of the progress of the campaign is advocated, an educational campaign aimed at explaining the life cycle of *E. granulosus*, the introduction of a system of government-appointed inspectors and a screen of the human population in the Falkland Islands, which may have been at risk of infection through contacts with sero-positive dogs, recommended.

Michael P. Reichel:

9. Zusammenfassung

Untersuchungen des epidemiologischen Status der Hydatidose/ Echinokokkose auf den Falkland Inseln.

Echinococcus granulosus, der dreigliedrige Hundebandwurm wird in den Falkland Inseln zum ersten Mal 1941 von Dr. Gibbs, dem damaligen Direktor des Departments for Agriculture, in seinem Jahresbericht erwähnt. Damals betrug die Prevalenz des zystischen *Echinococcus* in Schafen die in Darwin, auf Ostfalkland, geschlachtet wurden, 0.05%. 1953 wurden im Exportschlachthof in der Ajax Bay vom amtlichen Fleischbeschauer Zysten des Bandwurms schon in 3.3% der Lungen und Lebern der Schafe und Rinder festgestellt, 1954 waren *Echinococcus*-zysten nicht "selten". 1964 bemerkte dann ein, die Inseln besuchender Tierarzt, die hohe Prevalenz des Zystenstadiums in Schafen und Ende der 60iger Jahre fand ein weiterer Tierarzt, daß knapp 60% der Schafe und Rinder, die geschlachtet wurden, Zysten von *E. granulosus* beherbergten.

Menschliche Erkrankungen mit dem Metazestoden wurden dann bald ebenfalls bekannt und ein Kontrollprogramm zur Bekämpfung der Echinokokkose in die Wege geleitet. Die Bekämpfungskampagne führte rasch zum Abnehmen der Prevalenz in den Schafen, aber unterband auch scheinbar wirksam die weitere Übertragung auf den Menschen.

Anfang der neunziger Jahre gab es Anhaltspunkte dafür, daß die bis dahin weiter gesunkene Prevalenz des zystischen Stadiums des *E. granulosus* wiederum in der Zwischenwirtspopulation, den Schafen, zunahm. Daraufhin wurde ein Projekt geplant, daß mit neuerlich entwickelten, serologischen Methoden und Schlachthofuntersuchungen den epidemiologischen Status der Echinokokkose/Hydatidose in den Falkland Inseln bestimmen sollte.

Die Schlachthofuntersuchungen von Eingeweiden der Schafe am Schlachthof in Stanley und bei Hausschlachtungen auf den über 90 Farmen zeigten während des Untersuchungszeitraums von 1991 bis 1993 signifikante Prevalenzanstiege. Zysten von *E. granulosus* wurden deutlich häufiger am Schlachthof in Stanley festgestellt, bei tierärztlicher Untersuchung und signifikant häufiger in Schafen, die von Ostfalkländischen Farmen stammten, als von Farmen, die auf West Falkland gelegen waren. Die Prevalenz von *E. granulosus* Zysten am Schlachthof in Stanley stieg von

0.11% im Jahre 1991, über 0.29% 1992 auf 0.48% im Jahre 1993. Auf den Farmen wurden bei Hausschlachtungen jedoch nur 0.05% Zysten 1991 festgestellt, 0.006% 1992 und 0.04% 1993. Auf den Farmen wird die Beschau von Laienbeschauern durchgeführt was die signifikanten Prevalenzunterschiede sicher erklärt. Eine Echinokokkenzyste wurde 1992 in einem zweijährigem Schaf von Goose Green, Ost-Falkland gefunden, was darauf hindeutet, daß eine Übertragung von End- auf Zwischenwirt auch noch 1990 stattfand.

Die serologische Untersuchung der Blutproben von 908 Hunden aus den Falkland Inseln auf spezifische anti- *E. granulosus* Antikörper der IgG, IgA und IgE-Klassen per ELISA, ergab positive Untersuchungsergebnisse bei 16 Hunden (1.76%). Eine Behandlung mit Arecolinhydrobromid bei zwölf dieser Hunde konnte zwar keine adulten Echinokokken im Duodenalpurgativ darstellen, jedoch konnte auf Grund der geringen Sensitivität der Arecolinbehandlung die Anwesenheit von Adulten im Darm des Hundes nicht ausgeschlossen werden. Die positiven Hunde fanden sich in insgesamt 9 Lokalitäten, vier auf Westfalkland und fünf auf Ostfalkland. 11 Hunde waren testpositiv von Westfalkland, und fünf kamen von Ostfalkland. In einigen Lokalitäten fanden sich mehrere testpositive Hunde, und weitere Untersuchungen (Fragebogen, Begehung) deuteten auf mögliche Ursachen der Häufung hin. Nach einem Zwischenraum von drei bis zehn Monaten konnten zwölf der 16 positiven Hunden nochmals serologisch untersucht werden, wobei drei erneut ein positives Testresultat hatten. Von 61 weiteren mituntersuchten Hunden stammten sieben neuerdings positive Ergebnisse. Die Prevalenz einer anderen Tänie (*T. hydatigena*), die auch in diesem Studienzeitraum signifikant stieg, deutet daraufhin, daß die regelmäßige, sechswöchentliche Verabreichung von Praziquantel nicht von allen Hundebesitzern beachtet wird.

Fragebogenauswertung und Begehung der Farmen zeigten Mängel bei den Schlachtanlagen der meisten Farmen auf, die es Hunden ermöglichen, Zugang zu Schafeingeweiden und anderen Geweben zu erhalten. Damit ist die Möglichkeit der Übertragung und Infektion des Endwirtes mit Echinokokken gegeben. Viele der Farmer hatten auch nur rudimentäre Vorstellungen vom Kreislauf der Echinokokken und selber kaum Echinokokkenzysten in den letzten fünf Jahren gesehen. Auf einer Farm wurden Schaflebern und -lungen bevorzugt dadurch beseitigt, daß sie in die naheliegende See geworfen wurden. Die Häufung von testpositiven Hunden (vier) auf dieser Farm deutete daraufhin, daß diese Art der Schlachtabfallbeseitigung nicht geeignet war, den Lebenskreislauf des Parasiten wirksam zu unterbinden.

Diese Studie, die erste detaillierte der Echinokokkose auf den Falkland Inseln, deutet an, daß die Echinokokkose/Hydatidose noch immer auf den Inseln prevalent ist, und daß die Prevalenz steigt. Aus der Bewertung der Bekämpfungskampagne werden in dieser Studie Vorschläge für die weitere Durchführung der Echinokokkenbekämpfung auf den Falkland Inseln gemacht: der gezielten Aufklärung der Bevölkerung über den Lebenszyklus des Parasiten kommt eine zentrale Stellung in der weiteren Bekämpfungstrategie zu; die Schlachtanlagen auf den Farmen müssen dringend insoweit verbessert werden, daß den Hunden der Zugang zu Schlachtabfällen oder Schafgeweben unmöglich gemacht wird; staatliche Aufseher müssen die Einhaltung der relevanten Gesetzgebung garantieren, insbesondere die regelmäßige Praziquantelverabreichung auf den Farmen; und die serologische Erfolgsüberwachung der Kampagne per ELISA-Untersuchung (aber auch der Humanbevölkerung, die im Kontakt mit testpositiven Hunden stand) sollte fortgeführt werden, erweitert durch die gleichzeitige Anwendung von Copro-antigen assays.

10. References

1. ALLAN, J. C., G. AVILA, J. GARCIA NOVAL, A. FLISSER and P.S. CRAIG (1990):
Immunodiagnosis of taeniasis by coproantigen detection.
Parasitology 101, 473-477

2. ALLAN, J. C. and P. S. CRAIG (1989):
Coproantigens in gut tapeworm infections: Hymenolepis diminuta in rats.
Parasitol. Res. 76, 68-73

3. ALLAN, J. C., P. S. CRAIG, J. GARCIA NOVAL, F. MENCOS, D. LIU, Y. WANG, H. WEN, P. ZHOU, R. STRINGER, M. ROGAN and E. ZEYHLE (1992):
Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans.
Parasitology 104, 347-355

4. ALLAN, J. C., F. MENCOS, J. GARCIA-NOVAL, E. SARTI, A. FLISSER, Y. WANG, D. LIU and P. S. CRAIG (1993):
Dipstick dot ELISA for the detection of Taenia coproantigen in humans.
Parasitology 107, 79-85

5. ANON. (1949):
Dogs (Amendment) Ordinance 1949. Falkland Islands Gazette. The Secretariat. Port Stanley

6. ANON. (1965):
Tapeworm Eradication (Dogs) order. The Falkland Islands Gazette. 06 April 1965. The Secretariat. Port Stanley

7. ANON. (1970):
Tapeworm Eradication (Dogs) Order. The Falkland Islands Gazette. 28 January 1970. The Secretariat. Port Stanley

8. ANON. (1975):
Hydatid Eradication (Dogs) Order 1975. The Falkland Islands Gazette. The Secretariat. Port Stanley

9. ANON. (1981):
Hydatid Eradication (Dogs) Order 1981. The Falkland Islands Gazette. The Secretariat. Port Stanley

10. ANON. (1986):
Review of the Tasmanian Hydatid Limitation Programme. Hydatid Policy Review Committee. Department of Agriculture, Hobart, Tasmania

11. ANON. (1988):
Annual report. National Hydatids Council. Ministry of Agriculture and Fisheries, Wellington, New Zealand

12. ANON. (1992):
Annual Health Yearbook 1991, FAO-OIE-WHO. Rome, Italy

13. ANON. (1993):
Falkland Islands Farm Statistics 1992-1993. Secretariat. Port Stanley

14. ARUNDEL, J. H. (1972):
A review of cysticercoses of sheep and cattle in Australia.
Aust. Vet. J. 48, 140-155

15. BABOS, S. (1962):
Untersuchungen über die Serodiagnostik der Echinokokkose.
Angew. Parasitol. 3, 2-4

16. BÄHR, R. (1981):
Die Echinokokkose des Menschen.
Ferdinand Enke Verlag, Stuttgart

17. BALDOCK, F. C., R. C. A. THOMPSON, L. M. KUMARATILAKE and J. SHIELD (1985):
Echinococcus granulosus in farm dogs and dingoes in south eastern Queensland.
Aust. Vet. J. 62, 335-337

18. BARRIGA, O. O. and N. W. AL-KHALIDI (1991):
Effect of host sex and litter on the population dynamics of Echinococcus granulosus in dogs.
J. Parasitol. 77, 927-930

19. BEARD, T. C. (1969):
Hydatid control: a problem in health education.
Med. J. Aust. 2, 456-459

20. BEARD, T. C. (1973):
The elimination of echinococcosis from Iceland.
Bull. Wld. Hlth. Org. 48, 653-660

21. BEGG, N. C. (1961):
The campaign against hydatid disease: an experiment in social medicine.
N. Z. Med. J. 60, 229-234

22. BEGG, N. C., A. C. BEGG and R. G. ROBINSON (1957):
Primary hydatid disease of the brain - its diagnosis, radiological investigation, treatment and prevention.
N. Z. Med. J. 56, 84 - 98

23. BEVERIDGE, I. and M. D. RICKARD (1975):
The development of Taenia pisiformis in various definitive host species.
Int. J. Parasitol. 5, 633-639

24. BICKERS, M. M. (1970):
Hydatid disease of the female pelvis.
Am. J. Obstet. Gynecol. 107, 477-483

25. BIENENSTOCK, J. and A. D. BEFUS (1980):
Review, Mucosal Immunology.
Immunology 41, 241-270

26. BLAMIRE, R.V., R. H. GOODHAND and K. C. TAYLOR (1980):
A review of some animal diseases encountered at meat inspections in England and Wales, 1969 to 1978.
Vet. Rec. 106, 195-199

27. BLEANEY, A. (1984):
Hydatid disease in humans in the Falkland Islands 1984. 21 March 1984. Local Publication. Port Stanley.

28. BOWLES, J., D. BLAIR and D. P. MCMANUS (1992):
Genetic variants within the genus Echinococcus identified by mitrochondrial DNA sequencing.
Mol. Biochem. Parasitol. 54, 165-174

29. BRADFORD, M. M. (1976):
A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.
Anal. Biochem. 72, 248-254

30. BRAMBLE, A. J. (1974):
Hydatid disease control 1964-74.
Tas. J. Agric. Nov. 225-232

31. BRAMBLE, A. J. (1975):
Hydatid disease control in Tasmania...progress and new developments.
Tas. J. Agric. Nov. 231-236

32. BRYCESON, A. D. M., A. G. A. COWIE, C. MACLEOD, S. WHITE, D. EDWARDS, J. D. SMYTH and D. P. MCMANUS (1982):
Experience with mebendazole in the treatment of inoperable hydatid disease in England.
Trans. R. Soc. Trop. Med. Hyg. 76, 510-518

33. BURRIDGE, M. J. and C. W. SCHWABE (1977a):
Hydatid disease in New Zealand: an epidemiological study of transmission amongst Maoris.
Am. J. Trop. Med. Hyg. 26, 258-265

34. BURRIDGE, M. J. and C. W. SCHWABE (1977b):
Epidemiological analysis of factors influencing the rate of progress in Echinococcus granulosus control in New Zealand.
J. Hyg. 78, 151-163

35. BURRIDGE, M. J., C. W. SCHWABE and J. FRASER (1977a):
Hydatid disease in New Zealand: changing patterns in human infection, 1878-1972.
N. Z. Med. J. 85, 173-177

36. BURRIDGE, M. J., C. W. SCHWABE and T. W. PULLUM (1977b)
Path analysis: application in an epidemiological study of echinococcosis in New Zealand. J. Hyg. 78, 135-149

37. CAMERON, T. W. M. (1926):
Observations on the genus Echinococcus Rudolphi, 1801.
J. Helminthol. 4, 13-22

38. CAPRON, A., A. VERNES and J. BIGUET (1967):
Le diagnostic immunoéléctrophorétique de l'hydatidose. In: Le Kyste Hydatigue du foie. Lyon, SIMEP, 27-40

39. CHAKRAVARTI, D. N. and T. KRISTENSEN (1986):
Recent advances in the study of the molecular structure of the complement proteins.
Path. Immun. 5, 317-351

40. CHIN, D. D. (1981):
Hydatid cyst of the heart presenting as cerebral and cerebellar infarctions.
Med. J. Aust. 2, 556-557

41. CHORDI, A., J. GONZÁLES-CASTRO and Y. TORMO (1962):
Aportacion al estudio de las helminthiasis intestinales en los perros. II. Resultados de la prueba de floculación con Ben-thid y de la fijación de complemento en perros con Echinococcus granulosus.
Revta Ibér. Parasitol. 22, 285-290

42. CHORDI, A. and I. G. KAGAN (1965):
Identification and characterization of antigenic components of sheep hydatid fluid by immunoelectrophoresis.
J. Parasitol. 51, 63-71

43. COKER-VANN, M., P. BROWN and D. C. GAJDUSEK (1984):
Serodiagnosis of human cysticercosis using a chromatofocused antigenic preparation of Taenia solium cysticerci in an enzyme-linked immunosorbent assay (ELISA).
Trans. R. Soc. Trop. Med. Hyg. 78, 492-496

44. COLLI, C. W. and J. F. WILLIAMS (1972):
Influence of temperature on the infectivity of eggs of Echinococcus granulosus in laboratory rodents.
J. Parasitol. 58, 422-426

45. CRAIG, P. S., L. DESHAN and D. ZHAOXUN (1991):
Hydatid disease in China.
Parasitol. Today 7, 46-50

46. D'ALLESANDRO, A., R. L. RAUSCH, C. CUELLO and N. ARISTIZABAL (1979):
Echinococcus vogeli in man, with a review of polycystic hydatid disease in Colombia and neighboring countries.
Am. J. Trop. Med. Hyg. 28, 303-317

47. D'ALLESANDRO, A., R. L. RAUSCH, G. A. MORALES, S. COLLET and D. ANGEL (1981):
Echinococcus infections in Colombian animals.
Am. J. Trop. Med. Hyg. 30, 1263-1276

48. DAVIES, T. H., I. A. DICKSON, C. T. MCCREA, H. MEAD and W. W. WILLIAMS (1971):
The sheep and cattle industries of the Falkland Islands. Overseas Development Administration, London

49. DEPLAZES, P. and J. ECKERT (1988a):
Untersuchungen zur Infektion des Hundes mit Taenia hydatigena.
Schweiz. Arch. Tierheilkd. 128, 289-306

50. DEPLAZES, P. and J. ECKERT (1988b):
Massengewinnung und Lagerung von *Taenia hydatigena*-Eiern sowie Isolierung lebensfähiger Onkosphären.
Schweiz. Arch. Tierheilkd. **128**, 307-320

51. DEPLAZES, P., J. ECKERT, Z. S. PAWLOWSKI, L. MACHOWSKA and B. GOTTSTEIN (1991):
An ELISA for diagnostic detection of *Taenia saginata* coproantigens in humans.
Trans. R. Soc. Trop. Med. Hyg. **85**, 391-396

52. DEPLAZES, P., B. GOTTSTEIN, J. ECKERT, D. J. JENKINS, D. EWALD and S. JIMENEZ-PALACIOS (1992):
Detection of *Echinococcus* coproantigens by enzyme-linked immunosorbent assay in dogs, dingoes and foxes.
Parasitol. Res. **78**, 303-308

53. DEPLAZES, P., B. GOTTSTEIN, Y. STINGELIN and J. ECKERT (1990):
Detection of *Taenia hydatigena* coproantigens by ELISA in dogs.
Vet. Parasitol. **36**, 91-103

54. DE RYCKE, P. H. (1968):
The evagination of *Echinococcus granulosus* in media with different osmotic pressures.
Z. Parasitenkd. **30**, 192-198

55. DÉVÉ, F. (1910):
Echinococcose primitive expérimentale. Résistance des oeufs du échinocoque à la congélation.
C.R. Séanc. Soc. Biol. **69**, 568-570

56. DEW, H. R. (1926):
Observations on hydatid disease in the domestic herbivora (*Echinococcus multilocularis*) and its relationship with *Echinococcus alveolaris*.
Med. J. Aust. **2**, 301-309

57. DUNGAL, N. (1957):
Eradication of Hydatid disease in Iceland.
N. Z. Med. J. **56**, 213-222

58. DURAN, H., L. FERNANDEZ, F. GOMEZ-CASTESANA, L. LOPEZ-DURAN, P. MATA, D. BRANDAU and A. SANCHEZ-BARBA (1978):
Osseus hydatidosis.
J. Bone Joint Surg. 60-A **60**, 685-690

59. DURIE, P. H., AND R. F. RIEK (1952):
The role of the dingo and wallaby in the infestation of cattle with hydatids *Echinococcus granulosus* (Batsch 1786) Rudolphi, 1905, in Queensland.
Aust. Vet. J. **28**, 249-254

60. ECKERT, J. (1970):
Echinokokkose bei Mensch und Tier.
Schweiz. Arch. Tierheilkd. **112**, 443-457

61. ECKERT, J. (1981):
Echinokokkose.
Berl. Münch. Tierärztl. Wochenschr. **94**, 369-378

62. ECKERT, J., R. C. A. THOMPSON, A. J. LYMBERY, Z. S. PAWLOWSKI, B GOTTSTEIN and U. M. MORGAN (1993):
Further evidence for the occurrence of a distinct strain of *Echinococcus granulosus* in European pigs.
Parasitol. Res. **79,** 42-48.

63. FAY, F. H. (1973):
The ecology of Echinococcus multilocularis Leuckardt, 1863 (Cestoda: Taeniidae) on St Lawrence Island, Alaska. 1. Background and rationale.
Ann Parasitol. Hum. Comp. **48**, 523-542

64. FEATHERSTON, D. W. (1971):
Taenia hydatigena. II Evagination of cysticerci and establishment of adult stage in the dog.
Exp. Parasitol. **29**, 242-249

65. FESSLER, M. (1990):
Vergleich der Endemiegebiete von *Echinococcus multilocularis* und Tollwut in Mitteleuropa.
Vet. Diss. Zürich, Schweiz

66. FLETCHER, R. (1953):
Falkland Islands meat inspection report. Local publication. Archives. Port Stanley

67. FRANK, W. (1987):
Echinococcus multilocularis in Südwest-Deutschland - Persistenz einer Zoonose im Mitteleuropäischen Raum.
Heidelberger Geographische Arbeiten **83**, 86-113

68. FRENCH, C. M., G. S. NELSON and A. M. WOOD (1982):
Hydatid disease in the Turkana District of Kenya. I. The background to the problem with hypotheses to account for the remarkably high prevalence of the disease in man.
Ann. Trop. Med. Parasitol. **76**, 425-437,

69. GARABEDIAN, G. A., R. M. MATOSSIAN and A. Y. DJANIAN (1957):
An indirect haemagglutination test for hydatid disease.
J. Immunol. **78**, 269-272

70. GASSER, R. B., D. J. JENKINS, D. D. HEATH and S. B. LAWRENCE (1992):
Use of *Echinococcus granulosus* worm antigens for immunodiagnosis of *E. granulosus* infection in dogs.
Parasitology **45**, 89-100

71. GASSER, R. B., D. J. JENKINS, E. PAOLILLO, L. PARADA, P. CABRERA and P. S. CRAIG (1993):
Serum antibodies in canine echinococcosis.
Int. J. Parasitol. **23**, 579-586

72. GASSER, R. B., M. W. LIGHTOWLERS, D. L. OBENDORF, D. J. JENKINS and M. D. RICKARD (1988):
Evaluation of a serological test system for the diagnosis of natural *Echinococcus granulosus* infection in dogs using *E. granulosus* protoscolex and oncosphere antigen.
Aust. Vet. J. **65**, 369-373

73. GASSER, R. B., M. W. LIGHTOWLERS and M. D. RICKARD (1989):
Identification of protein components of *Echinococcus granulosus* protoscolex antigens for specific serodiagnosis of *E. granulosus* infection in dogs.
Parasite Immunol. **11**, 279-291

74. GASSER, R. B., M. W. LIGHTOWLERS and M. D. RICKARD (1990b):
A recombinant antigen with potential for serodiagnosis of *Echinococcus granulosus* infection in dogs.
Int. J. Parasitol. **20**, 943-950

75. GASSER, R. B., M. W. LIGHTOWLERS, M. D. RICKARD, R. A. LYFORD and H. J. S. DAWKINS (1990a):
Serological screening of farm dogs for *Echinococcus granulosus* infection in an endemic area.
Aust. Vet. J. **67**, 145-147

76. GEMMELL, M. A. (1957):
Hydatid disease in Australia. II. Observations on the geographical distribution of *Echinococcus granulosus* (Batsch, 1786) Rudolphi, 1805 in the dog in New South Wales.
Aust. Vet. J. **33**, 217-226

77. GEMMELL, M. A. (1958):
Cestode problems of domestic animals in the South Island of New Zealand.
N. Z. Med. J. **57**, 442-458

78. GEMMELL, M. A. (1959):
Hydatid disease in Australia. VI. Observations on the carnivora of New South Wales as definitive hosts of *Echinococcus granulosus* (Batsch, 1786) Rudolphi, 1805 and their role in the spread of hydatidiasis in domestic animals.
Aust. Vet. J. **35**, 450-455

79. GEMMELL, M. A. (1961):
An analysis of the incidence of hydatid cysts (*Echinococcus granulosus*) in domestic food animals in New Zealand.
N. Z. Vet. J. **9**, 29-37

80. GEMMELL, M. A. (1962):
Natural and acquired immunity factors interfering with developments during the rapid growth phase of *Echinococcus granulosus* in dogs.
Immunology **5**, 496-503

81. GEMMELL, M. A. (1973):
Surveillance of *Echinococcus granulosus* in dogs with arecoline hydrobromide.
Bull. Wld. Hlth. Org. **48**, 649-652

82. GEMMELL, M. A. (1975):
Report on the control programme directed against hydatidosis (*Echinococcus granulosus*) in the Falkland Islands. December 1975. The Secretariat. Local Publication. Port Stanley. Falkland Islands

83. GEMMELL, M. A. (1976):
Immunology and regulation of the cestode zoonoses.
In: S. COHEN and E SADUN (eds):
Immunology of parasitic infections.
Blackwell Scientific Publications, Oxford, 333-358

84. GEMMELL, M. A. (1977):
Taeniidae: modification to the life span of the egg and the regulation of tapeworm populations.
Exp. Parasitol. **41**, 314-328

85. GEMMELL, M. A. (1978):
The Styx-Field trial: Effect of treatment of the definitive host for tapeworms on larval forms in the intermediate host.
Bull. Wld. Hlth. Org. **56**, 433-443

86. GEMMELL, M. A. (1990):
Australasian contributions to an understanding of the epidemiology and control of hydatid disease caused by *Echinococcus granulosus* - past, present and future.
Int. J. Parasitol. **20**, 431-456

87. GEMMELL, M. A., and P. D. JOHNSTONE (1976):
Factors regulating tapeworm populations: dispersion of eggs of *Taenia hydatigena* on pasture.
Ann. Trop. Med. Parasit. **70**, 431-434

88. GEMMELL, M. A. and P. D. JOHNSTONE (1981):
Factors regulating tapeworm populations: estimations of the duration of acquired immunity by sheep to *Taenia hydatigena*.
Res. Vet. Sci. **30**, 53-56

89. GEMMELL, M. A., J. R. LAWSON and M. G. ROBERTS (1986):
Population dynamics in echinococcosis and cysticercosis: biological parameters of *Echinococcus granulosus* in dogs and sheep.
Parasitology **92**, 588-620

90. GEMMELL, M. A. and F. N. MCNAMARA (1976):
Factors regulating tapeworm populations: estimations of the infection pressure and index of clustering from *Taenia hydatigena* before and after removal of infected dogs.
Res. Vet. Sci. **21**, 215-219

91. GIBBS, J. G. (1946):
Agricultural Department report 1937-1946. The Secretariat. Port Stanley

92. GOLEMATIS, B. (1983):
Hydatid cyst of the pancreas: case report.
Mt. Sinai J. Med. **50**, 76-80

93. GOTTSTEIN, B. (1984):
An immunoassay for the detection of circulating antigens in human echinococcossis.
Am. J. Trop. Med. Hyg. **33**, 1185-1191

94. GOTTSTEIN, B. (1985):
Purification and characterisation of a specific antigen from *Echinococcus multilocularis*.
Parasite Immunol. **7**, 201-212

95. GOTTSTEIN, B., P. DEPLAZES, J. ECKERT, B. MUELLER, E. SCHOTT, O. HELLE, P. BOUJON, K., WOLFF A. WANDELER, U. SCHWIETE and H. MOEGLE (1991):
Serological (Em2-ELISA) and parasitological examination of fox populations for *Echinococcus multilocularis* infections.
Zentralbl. Veterinärmed. B. **38**, 161-168

96. GRABER, M. and J. THAL (1980):
L'échinococcose des artiodactyles sauvages de la République Centralafricaine: existence probable d'un cycle lion-phacochère.
Rev. Elev. Méd. Vét. Pays Trop. **33**, 51-59

97. GUBANOV, N. M. (1964):
Gel'mintofauna promyslovykh mlekopitaiushchikh Iakutii. Nauka. Moskva

98. HART, I. R. (1979):
The distribution of immunoglobulin-containing cells in canine small intestine.
Res. Vet. Sci. **27**, 269-274

99. HEATH, D. D. (1971):
The migration of oncospheres of *Taenia pisiformis, T. serialis* and *Echinococcus granulosus* within the intermediate host.
Int. J. Parasitol. **1**, 145-152

100. HEATH, D. D. (1973):
The life cycle of *Echinococcus granulosus*.
In: R. W. BROWN, J. R. SALISBURY and W. E WHITE,. (eds):
Recent advances in hydatid disease, Victoria, Australia.
Hamilton Medical and Veterinary Association, 7-18

101. HEATH, D. D. and S. B. LAWRENCE (1976):
Echinococcus granulosus: development *in vitro* from oncosphere to immature hydatid cyst.
Parasitology **73**, 417-423

102. HEATH, D. D., S. B. LAWRENCE, A. GLENNIE and H. TWAALFHOVEN (1985):
The use of excretory and secretory antigens of the scolex of *Taenia ovis* for the serodiagnosis of infection in dogs.
J. Parasitol.**71**, 192-199

103. HEATH, D. D., S. B. LAWRENCE and G. OUDEMANS (1988):
A blind test of serological response of dogs to infection with *Taenia ovis*.
N. Z. Vet. J. **36**, 143-145

104. HEINZ, H. J. and W. BRAUNS (1955):
The ability of flies to transmit ova of *Echinococcus granulosus* to human food.
S. Afr. J. Med. Sci. **20**, 131-132

105. HÖRCHNER, F., E. HAHN, M. HUWER and S. SANFT (1986):
Occurrence of hydatid cysts in Federal Republic of Germany. Internat. Congr. Inf. Dis. Parasitol. Munich. Abstr. No.1048. Futura-med Verlag. München

106. HOPE, M., J. BOWLES, P. PROCIV and D. P MCMANUS (1992):
A genetic comparison of human and wildlife isolates of *Echinococcus granulosus* in Queensland and the public health implications.
Med. J. Aust. **156**, 27-30

107. JACKSON, P. J. and J. H. ARUNDEL (1971):
The incidence of tapeworms in rural dogs in Victoria.
Aust. Vet J. **47**, 46-53

108. JAKUBOWSKI, M. S. and D. E. BARNARD (1971):
Anaphylactic shock during operation for hydatid disease.
Anaesthesiology **34**, 197-199

109. JENKINS, D. J., R. B. GASSER, T. ROMIG and E. ZEYHLE (1991):
Antibody response against natural *Taenia hydatigena* infection in dogs in Kenya.
Int. J. Parasitol. **21**, 251-253

110. JENKINS, D. J., R. B. GASSER, E. ZEYHLE, T. ROMIG and C. N. L. MACPHERSON (1990):
Assessment of a serological test for diagnosis of *Echinococcus granulosus* infection in dogs in Kenya.
Acta Trop. **47**, 245-248

111. JENKINS, D. J. and M. D. RICKARD (1984):
Haematological and serological data from dogs raised worm-free and monospecifically infected with helminths.
Aust. Vet. J. **61**, 309-311

112. JENKINS, D. J. and M. D. RICKARD (1985):
Specific antibody responses to *Taenia hydatigena, T. pisiformis* and *Echinococcus granulosus* in dogs.
Aust. Vet. J. **62**, 72-78

113. JENKINS, D. J. and M. D. RICKARD (1986):
Specific antibody responses in dogs experimentally infected with *Echinococcus granulosus*.
Am. J. Trop. Med. Hyg. **35**, 345-349

114. JOHNSON, J. S. and J. H. VAUGHAN (1967):
Canine immunoglobulins: I. Evidence of six immunoglobulin classes.
J. Immunol. **98**, 923-933

115. JOHNSON, J. S., J. H. VAUGHAN and S. N. SWISHER (1967):
Canine immunoglobulins: II Antibody activities in six immunoglobulin classes.
J. Immunol. **98**, 935-940

116. JOHNSTON, J. and R. B. GASSER (1993):
Copro-parasitological survey of dogs in Southern Victoria.
Aust. Vet. Pract. **23**, 127-131

117. KAGAN, I. G. (1968):
A review of serological tests for the diagnosis of hydatid disease.
Bull. Wld. Hlth. Org. **39**, 25-37

118. KILEJIAN, A., L. A. SCHINAZI and C. W. SCHWABE (1961):
Host-parasite relationships in echinococcosis. V. Histochemical observations on *Echinococcus granulosus*.
J. Parasitol. **47**, 1281-188

119. KILEJIAN, A. and C. W. SCHWABE (1971):
Studies on the polysaccharides of the *Echinococcus granulosus* cyst, with observations on a possible mechanism for laminated membrane formation.
Comp. Biochem. Physiol. **40B**, 25-36

120. KIRKLAND, K. (1966):
Urological aspects of hydatid disease.
Br. J. Urol. **38**, 241-254

121. KRABBE, H. (1864):
Observations for Icelanders on hydatids and precautions against them. Ministry of Justice, Copenhagen 1864. (Translation into English by I. R MAXWELL)
Aust. Vet. J. (1973), **49**, 395-401

122. KÜCHENMEISTER, G. F. H. (1852):
Ueber die Verwandlung der Finnen (Cysticerci) in Bandwürmer (Taenien).
Prag, Vierteljahrsschr., 106-158

123. KÜCHENMEISTER, G. F. H (1855):
Die in und an dem Körper des lebenden Menschen vorkommenden Parasiten. Ein Lehr- und Handbuch der Diagnose und Behandlung der thierischen und pflanzlichen Parasiten des Menschen. Leipzig.

124. KUMARATILAKE, L. M. and R. C. A. THOMPSON (1984):
Morphological characterisation of Australian strains of *Echinococcus granulosus*.
Int. J. Parasitol. **14**, 467-511

125. KUMARATILAKE, L. M., R. C. A. THOMPSON and J. D. DUNSMORE (1983):
Comparative strobilar development of *Echinococcus granulosus* of sheep origin from different geographical areas of Australia *in vivo* and *in vitro*.
Int. J. Parasitol. **13**, 151-156

126. KUMARATILAKE, L. M., R. C. A THOMPSON and J. ECKERT (1986):
Echinococcus granulosus of equine origin from different countries possess uniform morphological characteristics.
Int. J. Parasitol. **16**, 529-540

127. LAWS, G. F. (1968):
Physical factors influencing survival of taeniid eggs.
Exp. Parasitol. **22**, 227-239

128. LAWSON, J. R. and M. A. GEMMELL (1983):
Hydatidosis and cysticercosis: the dynamics of transmission.
Adv. Parasitol. **22**, 261-308

129. LAWSON, J. R. and M. A. GEMMELL (1985):
Dispersal of taeniid eggs with particular reference to the role of blowflies.
Parasitology **91**, 129-143

130. LEGGATT, G. R., W. YANG and D. P. MCMANUS (1992):
Serological evaluation of the 12kDa subunit of antigen B in *Echinococcus granulosus* cyst fluid by immunoblot analysis.
Trans. R. Soc. Trop. Med. Hyg. **86**, 189-192

131. LETHBRIDGE, R. C. (1980):
The biology of oncospheres of cyclophyllidean cestodes.
Helminth. Abstr. A. **49**, 59-72

132. LLOYD, S., S. C. MARTIN, T. M. H. WALTERS and E. J. L. SOULSBY (1991):
Use of sentinel lambs for early monitoring of the South Powys Hydatidosis Control Scheme.
Vet. Rec. **129**, 73-76

133. LYMBERY, A. J. and R. C. A. THOMPSON (1988):
Electrophoretic analysis of genetic variation in *Echinococcus granulosus* from domestic hosts in Australia.
Int. J. Parasitol. **18**, 803-811

134. MACDONALD, R. G. (1888):
Hydatid disease in New Zealand.
N. Z. Med. J. **2**, 80-89, 142-150

135. MACPHERSON, C. N. L. (1983):
An active intermediate host role for man in the life cycle of *Echinococcus granulosus* in Turkana, Kenya.
Am. J. Trop. Med. Hyg. **32**, 397-404

136. MACPHERSON, C. N. L., L. KARSTAD, P. STEVENSON and J. ARUNDEL (1983):
Hydatid disease in the Turkana district of Kenya. III. The significance of wild animals in the transmission of *Echinococcus granulosus*, with particular reference to Turkana and Masailand in Kenya.
Ann. Trop. Med. Parasitol. **77**, 61-73

137. MACPHERSON, C. N. L. and D. P. MCMANUS (1982):
A comparative study of *Echinococcus granulosus* from human and animal hosts in Kenya using isoelectric focusing and isoenzyme analysis.
Int. J. Parasitol. **12**, 515-521

138. MADELUNG, O. W. (1885):
Beiträge mecklenburgischer Ärzte zur Lehre von der Echinococcen-Krankheit.
Ferd. Enke Verlag, Stuttgart.

139. MARCHIONDO, A. A. and F. L. ANDERSEN (1983):
Fine structure and freeze-etch study of the protoscolex tegument of *Echinococcus multilocularis* (Cestoda).
J. Parasitol. **69**, 709-718

140. MATOFF, D. and G. KOLEV (1964):
The role of the hairs, muzzle and paws of echinococcic dogs in the epidemiology of echinococcosis.
Z. Tropenmed. Parasitol. **15**, 452-460

141. MATOSSIAN, R. M., M. L. MCLAREN, C. C. DRAPER, C. M. P. BRADSTREET, M. W. DIGHERO, G. J. KANE, L. M. MACKINLAY and M. D. RICKARD (1979):
The serodiagnosis of human hydatid disease. 2. Additional studies on selected sera using indirect haemagglutination (IHA), enzyme linked immunosorbent assay (ELISA), and defined antigen substrate spheres (DASS).
J. Helminthol. **53**, 287-291

142. MATOSSIAN, R. M., M. D. RICKARD and J. D. SMYTH (1977):
Hydatidosis: a global problem of increasing importance.
Bull. Wld. Hlth. Org. **55** 499-507

143. MCCONNELL, J. D. and R. J. GREEN (1979):
The control of hydatid disease in Tasmania.
Aust. Vet. J. **55**, 140-145

144. MCCREA, C. T. (1969):
Address to Executive Council. 02.December 1969. Minutes of meeting. Archives. Port Stanley

145. MCLAREN, M. L., J. E. LILLYWHITE and A. C. S. AU (1981):
Indirect enzyme-linked immunosorbent assay (ELISA): practical aspects of standardization and quality control.
Med. Lab. Sci. **38**, 245-251

146. MCMANUS, D. P. (1981):
A biochemical study of adult and cystic stages of *Echinococcus granulosus* of human and animal origin from Kenya.
J. Helminthol. **55**, 21-27

147. MCMANUS, D. P. and C. N. L. MACPHERSON (1984):
Strain characterization in the hydatid organism, *Echinococcus granulosus*: current status and new perspective.
Ann. Trop. Med. Parasitol. **28**, 193-198

148. MELDRUM, G. K. and J. D. MCCONNELL (1968):
The control of hydatid disease in Tasmania.
Aust. Vet. J. **44**, 212-217

149. MILLER, S. (1965):
The Hydatid cyst. A Falkland Islands Government Information Pamphlet. Local publication. Archives. Port Stanley.

150. MITCHELL, G. F. (1987):
Injection versus infection: the cellular immunology of parasitism.
Parasitol. Today **3**, 106-111

151. MORRIS, D. L. (1981):
Management of hydatid disease.
Br. J. Hosp. Med. June 1981, 586-590

152. MORRIS, D. L., P. W. DYKES, B. DICKSON, S. E. MARRINER, J. A. BOGAN and F. G. O. BURROWS (1983):
Albendazole in hydatid disease.
Br. Med. J. **286**, 103-104

153. MORRISON, P., R. STANTON and E. PILATTI (1988):
Echinococcus granulosus infection in wild dogs in south-eastern New South Wales.
Aust. Vet. J. **65**, 97-98

154. MOVSESIJAN, M. and Z. MLADENOVIC (1971):
Ispitivanje moguénosti koriscenja raznih razvojnih oblika *E. granulosus* u detekciji specificnih antitela prema istom parazitu. (The possibility of using different developmental stages of *E. granulosus* for detection of specific antibodies against this parasite)
Vet. Glasnik **25**, 159-163 (in croatian)

155. MOVSESIJAN, M., A. SOKOLIC and Z. MLADENOVIC (1968):
Studies on the immunological potentiality of irradiated *Echinococcus granulosus* forms: immunization experiments in dogs.
Br. Vet. J. **124**, 425-432

156. MUNRO, H. (1924):
Investigation into the conditions and practice of sheep farming in the Falkland Islands. Waterlow and Sons. London

157. NAUNYN, G. G. J. (1863):
Über die *Echinococcus hominis* zugehörige Tänie.
Archiv für Anatomie, Physiologie und wissenschaftliche Medizin, 412-416

158. NEWBY, T. J. and C. R. STOKES (1984):
Local immune responses of the gut. CRC Press, Boca Raton, Fla.

159. NOTT, D. B. (1979):
Hydatid disease in man - past, present and future.
Aust. Vet. J. **55**, 136-139

160. OKELO, G. B. A. (1986):
Hydatid disease: Research and control in Turkana. III. Albendazole in the treatment of inoperable hydatid disease in Kenya - a report on 12 cases.
Trans. R. Soc. Trop. Med. Hyg. **80**, 193-195

161. ORIEL, R., J. F. WILLIAMS, M.V. PÉREZ ESANDI and C. ORIEL (1971):
Purification of lipoprotein antigens of *Echinococcus granulosus* from sheep hydatid cyst.
Am. J. Trop. Med. Hyg. **20**, 569-574

162. PEREZ-GOMEZ, F., H. DURAN, S. TAMAMES, J. L. PERROTE and A. BLANES (1973):
Cardiac echinococcosis: clinical picture and complications.
Br. Heart. J. **35**, 1326-1331

163. POLYDOROU, K. (1976):
The control of the dog population in Cyprus as the first objective of the anti-*Echinococcus* campaign.
Bull. Off. Int. Epiz. **86**, 705-715

164. PURRIEL, P. P., P. M. SCHANTZ, H. BEOVIDE and G. MENDOZE (1973):
Human echinococcosis (hydatidosis) in Uruguay: a comparison of indices of morbidity and mortality, 1962-1971.
Bull. Wld. Hlth. Org. **49**, 395-402

165. RAUSCH,. R. L. (1954):
Studies on the helminth fauna of Alaska. XX. The histogenesis of the alveolar larva of *Echinococcus* species.
J. Infect. Dis. **94**, 178-186

166. RAUSCH, R. L. (1967):
On the ecology and distribution of *Echinococcus* spp. (Cestoda: Taeniidae) and characteristics of their development in the intermediate host.
Ann. Parasitol. Hum. Comp. **42**, 19-63

167. RAUSCH, R. L. (1975):
Taeniidae.
In: W. T. HUBBERT., W. F. MCCULLOCH and P. R. SCHURRENBERGER (eds): Diseases transmitted from animals to man.
Thomas, Springfield, Ill. 678-707

168. RAUSCH, R. L. (1986):
Life cycle patterns and geographic distribution of *Echinococcus* species.
In: R. C. A. THOMPSON (ed):
The Biology of *Echinococcus* and Hydatid disease.
George Allen and Unwin, London, 44-80

169. RAUSCH, R. L. and J. J. BERNSTEIN (1972):
Echinococcus vogeli sp. n. (Cestoda: Taeniidae) from the bush dog, *Speothos venaticus* (Lund).
Z. Trop. Med. Parasitol. **23**, 25-34

170. RAUSCH, R. L., A. D'ALESSANDRO and V.R. RAUSCH (1981):
Characteristics of the larval *Echinococcus vogeli* Rausch and Bernstein, 1972 in the natural intermediate host, the paca, *Cuniculus paca*. L. (Rodentia: Dasyproctidae).
Am. J. Trop. Med. Hyg. **30**, 1043-1052

171. RAUSCH, R. L. and S. H. RICHARDS (1971):
Observations on parasite-host relationships on *Echinococcus multilocularis* Leuckart, 1863 in North Dakota.
Can. J. Zool. **49**, 1317-1330

172. RAUSCH, R. L. and E. L. SCHILLER (1956):
Studies on the helminth fauna of Alaska. XXV. The ecology and public health significance of *Echinococcus sibiricensis* Rausch and Schiller, 1954 on St Laurence Island.
Parasitology **46**, 395-419

174. REICHEL, M. P. (1992):
The Importation of a national stud flock for the Falkland Islands.
VetScript, July 1992, New Zealand Veterinary Association

175. REYNOLDS, H. Y. and J. S. JOHNSON (1970):
Quantitation of canine immunoglobulins.
J. Immunol. **105**, 698-703

176. RICKARD, M. D. (1983):
Immunity.
In: C. ARME and P. W. PAPPAS (eds):
Biology of the Eucestoda.
Vol. 2 Academic Press, London

177. RICKARD, M. D. (1984):
Serological diagnosis and post-operative surveillance of human hydatid disease. I. Latex agglutination and immunoelectrophoresis using crude cyst fluid antigen.
Pathology **16**, 207-210

178. RICKARD, M. D., R.D. HONEY, J. L. BRUMLEY and G. F. MITCHELL (1984):
Serological diagnosis and post-operative surveillance of human hydatid disease. II. The enzyme-linked immunosorbent assay (ELISA) using various antigens.
Pathology **16**, 211-216

179. RIPPON, S.R. (1954):
Report of the Government Meat Inspector, Falkland Islands for 1954. Local publication. Archives. Port Stanley

180. ROITT, I. (1984):
Essential immunology. 5th ed. Blackwell Scientific Publications. Oxford

181. SAKAMOTO, I. and M. SUGIMURA (1970):
Studies on echinococcosis: XXIII. Electron microscopical observations on histogenesis of larval *Echinococcus multilocularis*.
Jpn. J. Vet. Res. **18**, 141- 147

182. SCHAEFER, J. (1984):
Survival of *Echinococcus multilocularis*-eggs in the natural environment and under laboratory conditions.
Zentralbl. Bakteriol. Mikrobiol. Hyg. I.A. **258**, 401-402

183. SCHANTZ, P. M. (1972a):
Localizacion de la hidatidosis en le sistema nerviosa central.
Bol. Of. Sanit. Panam. **63**, 198-202

184. SCHANTZ P. M. (1972b):
Hidatidosis: magnitud del problema y perspectivas de control.
Bol. Of. Sanit. Panam. **74**, 187-197

185. SCHANTZ, P. M. (1982):
Echinococcosis.
In: J. STEELE (ed):
CRC handbook series in zoonoses, Section C: Parasitic zoonoses. **1**, 231-277
Boca Raton, Fla, CRC Press

186. SCHANTZ, P. M. and C. COLLI (1973):
Echinococcus oligarthrus (Diesing, 1863) from Geoffroy's cat (*Felis geoffroyi* d'Orbigny y Gervats) in temperate South America.
J. Parasitol. **59**, 1138-1140

187. SCHANTZ, P. M., B. GOTTSTEIN, R. AMMANN and A. LANIER (1991):
Hydatid and the Arctic.
Parasitol. Today **7**, 35-36

188. SCHANTZ, P. M. and C.W. SCHWABE (1969):
Worldwide status of hydatid disease control.
J. Am. Vet. Med. Assoc. **155**, 2104-2121

189. SCHANTZ, P. M., J. F. WILLIAMS and C. RIVA POSSE (1973):
The epidemiology of hydatid disease in southern Argentina. Comparison of morbidity indices, evaluation of immunodiagnostic tests and factors affecting transmission in southern Rio Negro province.
Am. J. Trop. Med. Hyg. **22**, 629-641

190. SCHILLER, E. L. (1954):
Studies on the helminth fauna of Alaska. XIX. An experimental study on blowfly (*Phormia regina*) transmission of hydatid disease.
Exp. Parasitol. **3**, 161-166

191. SCHILLER, E. L. (1955):
Studies on the helminth fauna of Alaska. XXVI. Some observations on the cold-resistance of eggs of *Echinococcus sibiricensis* Rausch and Schiller, 1954.
J. Parasitol. **41**, 578-582

192. SCHWABE, C. W. (1984):
Veterinary medicine and human health. 3ed., Williams and Wilkins, Baltimore

193. SCHWABE, C. W. (1986):
Current status of hydatid disease: a zoonosis of increasing importance.
In: R. C. A. THOMPSON (ed):
The Biology of Echinococcus and Hydatid disease.
George Allen and Unwin, London, 81-113

194. SCHWABE, C. W., L. A. SCHINAZI and A. KILEJIAN (1959):
Host-parasite relationships in echinococcosis. II. Age resistance to secondary echinococcosis in the white mouse.
Am. J. Trop. Med. Hyg. **8**, 29-36

195. SCHWARTZ, A. and J. M. KEHOE (1983):
Fundamental principles of immunology.
In: S. J. ETTINGER. (ed):
Textbook of veterinary internal medicine. Diseases of the dog and cat. Vol.2.
W. B. Saunders, Philadelphia

196. SHAMSUL ISLAM, A. W. M. (1979):
Hydatid disease in sheep in Mymensingh District, Bangladesh.
J. Parasitol. **65**, 37

197. SHEPHERD, J. C. and D. P. MCMANUS (1987):
Specific and cross-reactive antigens of *Echinococcus granulosus* hydatid cyst fluid.
Mol. Biochem. Parasitol. **25**, 143-154

198. SINGH, B. P. and D. N. DHAR (1988):
Indirect fluorescent antibody test for the detection of antibodies to *Echinococcus granulosus* in experimentally infected pups.
Vet. Parasitol. **28**, 185-190

199. SLAIS, J. (1973):
Functional morphology of cestode larvae.
Adv. Parasitol. **11**, 395-480

200. SMYTH, J. D. (1964):
The biology of the hydatid organism.
Adv. Parasitol. **2**, 169-219

201. SMYTH, J. D. (1967):
Studies on tapeworm physiology. XI. *In vitro* cultivation of *Echinococcus granulosus* from the protoscolex to the strobilate stage.
Parasitology **57,** 11-133

202. SMYTH, J. D. (1969):
The physiology of cestodes.
Oliver and Boyd, Edinburgh

203. SMYTH, J. D. (1977):
Strain differences in *Echinococcus granulosus*, with special reference to the status of equine hydatidosis in the United Kingdom.
Trans. R. Soc. Trop. Med. Hyg. **71**, 93-100

204. SMYTH, J. D. (1982):
Speciation in *Echinococcus*: biological and biochemical criteria.
Revta. Iber. Parasitol. Vol. Extra, 25-34

205. SMYTH, J. D. and Z. DAVIES (1974):
Occurrence of physiological strains of *Echinococcus granulosus* demonstrated by *in vitro* culture of protoscoleces from sheep and horse hydatid cysts.
Int. J. Parasitol. **4**, 443-445

206. SMYTH, J. D., M. A. GEMMELL and M. M. SMYTH (1969):
Establishment of *Echinococcus granulosus* in the intestine of normal and vaccinated dogs.
Ind. J. Helminthol. Srivastava Commerative Volume, 167-178

207. SMYTH, J. D. and M. M. SMYTH (1969):
Self insemination in *Echinococcus granulosus in vivo.*
J. Helminthol. **43**, 383-388

208. SOUSA, O. E. and V. E. THATCHER (1969):
Observations on the life-cycle of *Echinococcus oligarthrus* (Diesing, 1863) in the Republic of Panama.
Ann. Trop. Med. Parasitol. **63**, 165-175

209. SPRUANCE, S. L. (1974):
Latent period of 53 years in a case of hydatid cyst disease.
Arch. Intern. Med. **134**, 741-742

210. STORANDT, S. T. and K. R. KAZACOS (1993):
Echinococcus multilocularis identified in Indiana, Ohio, and East-central Illinois.
J. Parasitol. **79**, 301-305

211. SWEATMAN, G. K. and R. J. WILLIAMS (1963):
Survival of *Echinococcus granulosus* and *Taenia hydatigena* eggs in two extreme climatic regions of New Zealand.
Res. Vet. Sci. **4**, 199-216

212. SWEATMAN, G. K., R. J. WILLIAMS, K. M. MORIARTY and T. C. HENSHALL (1963):
On acquired immunity to *Echinococcus granulosus* in sheep.
Res. Vet. Sci. **4**, 187-198

213. SWIDERSKI, Z. (1982):
Echinococcus granulosus: embryonic envelope formation.
Proc. 10th Int. Cong. Electron Microsc. **3**, 513

214. THIENPONT, D., F. ROCHETTE and O. F. J. VANPARIJS (1986):
Diagnosing helminthiasis by coprological examination. Janssen Research Foundation. Beerse, Belgium

215. THOMAS, J. D. (1881):
The statistics of hydatid disease in the Australian colonies.
Aust. Med. J. **3**, 344-348

216. THOMPSON, R. C. A. (1975):
Studies on equine hydatidosis in Great Britain. PhD thesis. University of London

217. THOMPSON, R. C. A. (1977):
Growth, segmentation and maturation of the British horse and sheep strain of *Echinococcus granulosus* in dogs.
Int. J. Parasitol. **7**, 281-285

218. THOMPSON, R. C. A. (1979):
Biology and speciation of *Echinococcus granulosus*.
Aust. Vet. J. **55**, 93-98

219. THOMPSON, R. C. A. (1983):
The susceptibility of the European red fox (*Vulpes vulpes*) to infection with *Echinococcus granulosus* of Australian sheep origin.
Ann. Trop. Med. Parasitol. **77**, 75-82

220. THOMPSON, R. C. A. (1986):
Biology and systematics of *Echinococcus*.
In: R. C. A THOMPSON. (ed):
The biology of Echinococcus and hydatid disease.
George Allen and Unwin, London, 5-43

221. THOMPSON, R. C. A., J. D. DUNSMORE and A. R. HAYTON (1979):
Echinococcus granulosus: secretory activity of the rostellum of the adult cestode *in situ* in the dog.
Exp. Parasitol. **48**, 144-163

222. THOMPSON, R. C. A. and J. ECKERT (1982):
The production of eggs by *Echinococcus granulosus* in the laboratory following *in vivo* and *in vitro* development.
Z. Parasitenkd. **68**, 227-234

223. THOMPSON, R. C. A. and J. ECKERT (1983):
Observations on *Echinococcus multilocularis* in the definitive host.
Z. Parasitenkd. **69**, 335-345

224. THOMPSON, R. C. A. and L. M. KUMARATILAKE (1982):
Intraspecific variation in *Echinococcus granulosus*: the Australian situation and perspectives for the future
Int. J. Parasitol. **14**, 283-291

225. THOMPSON, R. C. A. and L. M. KUMARATILAKE (1985):
Comparative development of Australian strains of *Echinococcus granulosus* in dingoes (*Canis familiaris dingo*) and domestic dogs (*C. f. familiaris*) with further evidence gor the origin of he sylvatic strain.
Int. J. Parasitol. **15**, 535-542

226. THOMPSON, R. C. A., L. M. KUMARATILAKE and J ECKERT (1984):
Observations on *Echinococcus granulosus* of cattle origin in Switzerland.
Int. J. Parasitol. **14**, 283-291

227. THOMPSON, R. C. A. and A. J. LYMBERY (1988):
The nature, extent and significance of variation within the genus *Echinococcus*.
Adv. Parasitol. **27**, 209-258

228. THOMPSON, R. C. A. and A. J. LYMBERY (1990a):
Intraspecific variation in parasites - what is a strain?
Parasitol. Today **6**, 345-348

229. THOMPSON, R. C. A. and A. J. LYMBERY (1990b):
Echinococcus: Biology and strain variation.
Int. J. Parasitol. **20**, 457-470

230. THOMPSON, R. C. A., D. B. NOTT, J. SQUIRE and D. RENNELL (1987):
Evidence that the Australian sylvatic strain of *Echinococcus granulosus* is infective to humans.
Med. J. Aust. **146**, 396-397

231. THOMPSON, R. C. A., I. D. ROBERTSON, R. B. GASSER and C. C. CONSTANTINE (1993):
Hydatid disease in Western Australia: a novel approach to education and surveillance.
Parasitol. Today **9**, 431-433

232. THOMPSON, R. C. A. and J. D. SMYTH (1975):
Equine hydatidosis: a review of the current status in Great Britain and the results of an epidemiological survey.
Vet. Parasitol. **1**, 107-127

233. TIZARD, I. (1992):
Veterinary Immunology-an introduction. 4th. ed. W.B.Saunders Company

234. TORGERSON, P. R., F. M. D. GULLAND and M. A. GEMMELL (1992):
Observations on the epidemiology of *Taenia hydatigena* in Soay sheep on St. Kilda.
Vet. Rec. **131**, 218-219

235. TURNER, E. L., D. A. BERBERIAN and E. W. DENNIS (1933):
Successful artificial immunization of dogs against *Taenia echinococcus*.
Proc. Soc. Exp. Biol. Med. **30**, 618-619

236. ,TURNER, E. L., E. W. DENNIS and D. A. BERBERIAN (1935):
The value of the Casoni test in dogs.
J. Parasitol. **21**, 180-182

237. VAERMAN, J. P., P. A. CRABBE, D. R. NASH and J. F. HEREMANS (1970):
Quantitative and immunochemical studies on dog's immunoglobulins.
Progr. Immunobiol. **4**, 193-198

238. VARELA-DÍAZ, V. M., E. A. COLTORI, U. PREZIOSO, M. H. LÓPEZ-LEMES, J. A. GUISANTES and L. A. YARZÁBAL (1975):
Evaluation of three immunodiagnostic tests for human hydatid disease.
Am. J. Trop. Med. Hyg. **24**, 312-319

239. VIRCHOW, R. (1855):
Die multiloculäre, ulcerirende Echinokokkengeschwulst der Leber.
Verh. Physiol.-Med. Ges. Würzburg **6**, 428-429

240. VIZARD, A. L., G. A. ANDERSON and R.B. GASSER (1990):
Determination of the optimum cut-off value for a diagnostic test.
Prev. Vet. Med. **10**, 137-143

241. VOGEL, H. (1961):
Biologie und Parasitologie des Alveolarechinokokkus.
Chir. Praxis. **5**, 407-412

242. VON FELLENBERG, R. (1978):
Kompendium der allgemeinen Immunologie.
Paul Parey, Berlin and Hamburg

243. VON SIEBOLD, C. T. E. (1853):
Über die Verwandlung der *Echinococcus*-Brut in Taenien.
Z. Wiss. Zool. **4**, 409-425

244. VUITTON, D. A. (1990):
Alveolar echinococcosis of the liver: a parasitic disease in search of a treatment.
Hepatology **12**, 617-618

245. WACHIRA, T. M., C. N. L. MACPHERSON and J. M. GATHUMA (1990):
Hydatid disease in the Turkana district of Kenya. VII. Analysis of the infection pressure between definitive and intermediate hosts of *Echinococcus granulosus* 1979-1988.
Ann. Trop. Med. Parasitol. **84**, 361-368

246. WACHIRA, T. M., C. N. L. MACPHERSON and J. M. GATHUMA (1991):
Release and survival of *Echinococcus* eggs in different environments in Turkana, and their possible impact on the incidence of hydatidosis in man and livestock.
J. Helminth. **65**, 55-61

247. WAKELIN, D. (1985):
Genetic control of immunity to helminth infections.
Parasitol. Today **1**, 17-23

248. WALTERS, T. M. H. and M. J. CLARKSON (1980):
The development of *Echinococcus granulosus* in sheep dogs, beagles and foxes.
Trans. R. Soc. Trop. Med. Hyg. **74**, 118

249. WATSON-JONES, D. L. and C. N. L. MACPHERSON (1988):
Hydatid disease in the Turkana district of Kenya. VI. Man:dog contact and its role in the transmission and control of hydatid disease amongst the Turkana.
Ann. Trop. Med. Parasitol. **82**, 343-356

250. WHITACRE, C. and R. W. LAND (1980):
DEAE cellulose chromatographic characterization of canine serum.
Comp. Immunol. Microbiol. Infect. Dis. **3**, 525-534

251. WHITLEY, R. S. (1983):
Veterinary Research and Disease Control in The Falkland Islands 1976-1983.
Overseas Development Administration, London

252. WILHELM, O. (1953):
La hidatidosis equinococosica en la provencia de Concepción (Chile).
Arch. Int. Hydatidosis **13**, 379-392

253. WILLARD, M. C., J. E.COOK, L. S. RODKEY, A. D. DAYTON and N. V. ANDERSON (1978):
Morphologic evaluation of IgM cells of the canine small intestine by fluorescence microscopy.
Am. J. Vet. Res. **39**, 1502-1505

254. WILLIAMS, J. F. and M. V. PÉREZ ESANDI (1971):
Reaginic antibodies in dogs infected with *Echinococcus granulosus*.
Immunology **20**, 451-455

255. WILLIAMS, J. F. and G. K. SWEATMAN (1963):
On the transmission, biology and morphology of *Echinococcus granulosus equinus*, a new subspecies of hydatid tapeworms in horses in Great Britain.
Parasitology **53**, 391-407

256. WILLIS, J, I. V. HERBERT and T. C. ELLIOTT (1981):
Some factors affecting the survival and transmission of *Taenia multiceps* eggs.
Proc. 9th Int. Conf. W.A.A.V.P. Budapest, 126

257. WILSON, J. F., A. C. DIDDAMS and R. L. RAUSCH (1968):
Cystic hydatid disease in Alaska. A review of 101 autochthonous cases of *Echinococcus granulosus* infection.
Am. Rev. Resp. Dis. **98**, 1-15

258. WORBES, H. (1986):
Diagnostische Probleme einer Echinokokkeninfektion im Raum Thüringen.
11. Haupttagung Parasitol. Ges. DDR Magdeburg.

259. YAMASHITA, J., M. OHBAYASHI and S. KONNO (1956):
Studies on echinococcosis: III. On experimental infections in dogs, especially on the development of *Echinococcus granulosus* (Batsch, 1786).
Jpn. J. Vet. Res. **4**, 113-122

260. ZEYHLE, E. (1982):
Die Verbreitung von *Echinococcus multilocularis* in Südwestdeutschland.
In: R. BÄHR (ed):
Probleme der Echinokokkose unter Berücksichtigung parasitologischer und klinischer Aspekte.

Hans Huber Verlag, Bern, 26-33

11. Acknowledgments

Thanks are due to Dr Robin Gasser, Senior Lecturer in Veterinary Parasitology at the University of Melbourne, for his support throughout this project, initially in the Falkland Islands and later during my stay in Melbourne. I am also grateful to Dr Thomas Schnieder (Privatdozent, Tierärztliche Hochschule Hannover) for his support and acceptance of this project thesis for submission in fulfilment of the requirements of the degree of *Doctor medicinae veterinariae*.

In the Falkland Islands the support and cooperation of all farmers and dog owners is specifically acknowledged. Without their support and willing submission of their dogs for blood testing, this study would not have been possible. I am particularly grateful to the proprietors of the Stanley abattoir, Messrs. Jones and Berntsen for their assistance during the collection of sheep offal inspection data. Mr David Baber, Senior Laboratory Technician in Stanley assisted with and gave technical support during the collection of blood samples and their preparation for dispatch to Melbourne. David also helped with the laboratory preparation of cyst material for examination in Stanley and in Auckland, New Zealand. In Melbourne, Angus Campbell, Bachelor of Animal Science student, assisted with the ELISA testing of some Falkland Islands dog sera. Thanks are due to Drs Keith McSporran and Derek Belton of the Lynfield Animal Health Laboratory in Auckland for histological examination of samples. Mrs Maggie Barkmann and Mrs Mandy McLeod assisted with secretarial work and with the inspection of sheep offal at the Stanley abattoir and the recording of cysts. Thanks also to Ms Jane Cameron, archivist in Stanley for help with locating documents on the history of *E. granulosus* in the Islands. Dr Marshall Lightowlers, Melbourne University, helped with his critical comments in the preparation of manuscript. Dr Dave Obendorf, parasitologist, Department of Primary Industries and Energy at the Mount Pleasant Laboratories in Launceston, Tasmania is due thanks for his information and discussions about the Tasmanian hydatid eradication campaign, and Prof Philip Craig, University of Salford, Manchester for his discussions on aspects of *E. granulosus* eradication and copro-antigen assays.

Appendices

Appendix 1

FALKLAND ISLANDS

DOGS ORDINANCE
(Chapter 21)

Hydatid Eradication (Dogs) Order 1981

No. 3 of 1981.

R. M. HUNT.
Governor.

IN EXERCISE of the powers conferred by section 12A of the Dogs Ordinance, the Governor has made the following order —

1. This order may be cited as the Hydatid Eradication (Dogs) Order 1981 and shall come into operation on the 1st day of July 1981.

2. In this order, unless the context otherwise requires —
"carcass" means the skinned or unskinned body of an herbivorous animal;
"herbivorous animal" shall include sheep, pigs, cattle, horses and guanaco.

3. The Governor may appoint a Chief Inspector and any number of Inspectors for the purpose of this order.

4. The owner or any person in charge of a dog shall be supplied, at cost price, only with such doses of a preparation as may be obtained from and administered by or under the direction of an Inspector or a resident Veterinary Surgeon and which shall be administered to the dog in his charge at such intervals and in such manner as specified by the Governor in Council.

5. An Inspector shall have the power to inspect any dog at any reasonable time.

6. The owner or any person in charge of a dog shall ensure that it is confined or securely tethered unless being worked or exercised under direct supervision.

7. The owner or any person in charge of a dog shall ensure that it is kept in a proper state of health and cleanliness.

8. Within the area of a settlement no carcass of any herbivorous animal shall be opened except in a place which is constructed in such a way as to prevent access by dogs and which has a drain constructed in such a way as to deny access to dogs, cats and birds. At an outside shepherd's house or other place outside a settlement, no carcass shall be opened except in a place as defined in the foregoing sentence without the written permission of the Chief Inspector. If the owner, lessee or tenant of any premises wishes to slaughter any herbivorous animal, he shall be liable to provide facilities to comply with this provision without delay and in any event within twelve months of the coming into operation of this order.

9. When an extraordinary number of herbivorous animals are slaughtered, the carcasses shall be stacked either in a dog-proof enclosure for a minimum of 28 days or at a place which has the written approval of the Chief Inspector.

10. No person shall feed or allow to be fed to any dog any liver, lung or heart of an herbivorous animal, nor shall any person allow any dog access to such liver, lung or heart of such animal.

11. Any person who opens the carcass of an herbivorous animal shall remove the liver, lungs and heart and shall dispose of them within an area to which access to dogs is prevented, preferably by burning to ash or by any other way approved in writing by the Chief Inspector.

12. It shall be the duty of any person who knows of a dead herbivorous animal within half a mile of a dwelling house to report its whereabouts without delay to the person responsible who shall, as soon as is practicable, arrange for the permanent disposal of such animal in such a way as to deny access to dogs.

13. The Governor in Council may grant special dispensation from any of the provisions of this order in certain circumstances.

14. The Chief Inspector or any Police Officer may, for the purpose of ascertaining adherence to the provisions of this order, at all reasonable times enter any land or premises.

15. Any person who obstructs or impedes any Police Officer or Inspector in the execution of his duty or contravenes any of the provisions of this order, shall commit an offence and shall be liable on summary conviction to a fine not exceeding £200 for a first offence or £500 for a second or each subsequent offence.

16. The Hydatid Eradication (Dogs) Order 1975 is cancelled.

13th May 1981.

By Command,
F. E. BAKER,
Chief Secretary.

Ref. AGR/10/4.

Appendix 2

Farm Questionnaire
Falkland Islands 1993

Name or number of dog under investigation?

Age: Sex:

Name and address of property:

1. How long has farmer/owner been on the property?

2. Were any sheep bought in?

3. From where?

Size of farm

Do other farmers/dogs pass through? Yes/No

General state of property: Very tidy/Average/Untidy

How many sheep on the farm?

Dogs on the property

Number of dogs: Working:. Retired:

Who does praziquantel dosing?

How long has dog been on the farm?

Has dog been on other farms?

What happens if owner away gathering/town at dog dosing?

History of *E.granulosus*/*T.hydatigena* infection in sheep:

Home killing

Who does it?

How many sheep a week/month/year?

Where is the killing done?

Is the site dog-proof?

Drain/sump present?

Offal disposal?

Dog Feeding

Who usually feeds dogs?

How often are dogs fed?

Source of dog food?

Dog Control

Any untreated dogs/pups?

Latest new dog arrival on the farm:

Dog housing:

Dogs tied up when not in use?

Dogs loose at time of visit:

Stray dogs: Yes/No

Hydatid knowledge

Has owner ever seen hydatid cyst?

Has owner ever seen bladder cyst?

What does owner know about life cycle of *E. granulosus*?

Mass killings Yes/No

When?

How far from the settlement? Offal disposal?

Additional notes:

	A	B	C	D	E	F	G	H	I
1	IgG	IgA	IgE	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
2	0.308	0.286	-0.003	-0.253	-0.247	-0.273			
3	0.121	0.110	-0.003	-0.440	-0.423	-0.273			
4	0.256	0.156	-0.003	-0.305	-0.377	-0.273			
5	0.036	0.063	0.010	-0.525	-0.470	-0.260			
6	0.045	0.071	0.006	-0.516	-0.462	-0.264			
7	0.031	0.078	0.003	-0.530	-0.455	-0.267			
8	0.039	0.067	0.000	-0.522	-0.466	-0.270			
9	0.047	0.060	0.000	-0.514	-0.473	-0.270			
10	0.057	0.071	0.013	-0.504	-0.462	-0.257			
11	0.078	0.111	-0.010	-0.483	-0.422	-0.280			
12	0.093	0.092	-0.003	-0.468	-0.441	-0.273			
13	0.080	0.094	0.000	-0.481	-0.439	-0.270			
14	0.051	0.093	0.019	-0.510	-0.440	-0.251			
15	0.053	0.090	0.003	-0.508	-0.443	-0.267			
16	0.070	0.089	-0.003	-0.491	-0.444	-0.273			
17	0.066	0.087	0.000	-0.495	-0.446	-0.270			
18	0.092	0.148	0.006	-0.469	-0.385	-0.264			
19	0.118	0.070	-0.010	-0.443	-0.463	-0.280			
20	0.138	0.069	0.006	-0.423	-0.464	-0.264			
21	0.067	0.093	-0.003	-0.494	-0.440	-0.273			
22	0.180	0.317	-0.006	-0.381	-0.216	-0.276			
23	0.162	0.084	0.006	-0.399	-0.449	-0.264			
24	0.085	0.086	-0.003	-0.476	-0.447	-0.273			
25	0.161	0.089	-0.003	-0.400	-0.444	-0.273			
26	0.082	0.104	0.000	-0.479	-0.429	-0.270			
27	0.044	0.050	-0.006	-0.517	-0.483	-0.276			
28	0.072	0.053	-0.003	-0.489	-0.480	-0.273			
29	0.108	0.167	-0.006	-0.453	-0.366	-0.276			
30	0.116	0.017	0.006	-0.445	-0.516	-0.264			
31	0.087	0.158	0.086	-0.474	-0.375	-0.184			
32	0.136	0.290	0.003	-0.425	-0.243	-0.267			
33	0.156	0.122	0.246	-0.405	-0.411	-0.024			
34	0.147	0.203	0.022	-0.414	-0.330	-0.248			
35	0.170	0.177	0.147	-0.391	-0.356	-0.123			
36	0.137	0.186	0.198	-0.424	-0.347	-0.072			
37	0.237	0.274	0.150	-0.324	-0.259	-0.120			
38	0.226	0.187	0.115	-0.335	-0.346	-0.155			
39	0.274	0.110	0.016	-0.287	-0.423	-0.254			
40	0.392	0.239	0.006	-0.169	-0.294	-0.264			
41	0.349	0.301	-0.003	-0.212	-0.232	-0.273			
42	0.174	0.218	0.000	-0.387	-0.315	-0.270			
43	0.250	0.091	0.019	-0.311	-0.442	-0.251			
44	0.178	0.157	0.032	-0.383	-0.376	-0.238			
45	0.173	0.281	-0.003	-0.388	-0.252	-0.273			
46	0.221	0.166	0.025	-0.340	-0.367	-0.245			
47	0.179	0.116	0.048	-0.382	-0.417	-0.222			
48	0.171	0.175	0.029	-0.390	-0.358	-0.241			
49	0.296	0.263	0.010	-0.265	-0.270	-0.260			
50	0.301	0.259	0.045	-0.260	-0.274	-0.225			
51	0.221	0.141	0.022	-0.340	-0.392	-0.248			
52	0.243	0.273	0.096	-0.318	-0.260	-0.174			
53	0.146	0.167	-0.003	-0.415	-0.366	-0.273			
54	0.190	0.197	0.080	-0.371	-0.336	-0.190			
55	0.388	0.336	0.045	-0.173	-0.197	-0.225			
56	0.360	0.294	0.000	-0.201	-0.239	-0.270			
57	0.378	0.280	0.045	-0.183	-0.253	-0.225			
58	0.276	0.165	0.003	-0.285	-0.368	-0.267			
59	0.134	0.152	0.160	-0.427	-0.381	-0.110			
60	0.310	0.145	0.064	-0.251	-0.388	-0.206			
61	0.181	0.161	0.074	-0.380	-0.372	-0.196			
62	0.157	0.211	0.147	-0.404	-0.322	-0.123			
63	0.220	0.255	0.138	-0.341	-0.278	-0.132			
64	0.105	0.200	0.115	-0.456	-0.333	-0.155			
65	0.101	0.220	0.105	-0.460	-0.313	-0.165			
66	nd	0.156	0.134	-0.561	-0.377	-0.136			
67	0.115	0.145	0.019	-0.446	-0.388	-0.251			
68	0.072	0.104	0.079	-0.489	-0.429	-0.191			
69	0.052	0.120	0.007	-0.509	-0.413	-0.263			
70	0.098	0.132	0.023	-0.463	-0.401	-0.247			
71	0.009	0.083	0.003	-0.552	-0.450	-0.267			
72	0.190	0.170	0.118	-0.371	-0.363	-0.152			
73	0.043	0.239	0.003	-0.518	-0.294	-0.267			
74	0.024	0.128	0.001	-0.537	-0.405	-0.269			

	A	B	C	D	E	F	G	H	I
75	IgG	IgA	IgE	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
76	0.110	0.081	0.029	-0.451	-0.452	-0.241			
77	0.268	0.114	0.001	-0.293	-0.419	-0.269			
78	0.122	0.203	0.011	-0.439	-0.330	-0.259			
79	0.159	0.160	0.119	-0.402	-0.373	-0.151			
80	0.112	0.287	0.019	-0.449	-0.246	-0.251			
81	0.102	0.079	0.044	-0.459	-0.454	-0.226			
82	0.366	0.112	0.014	-0.195	-0.421	-0.256			
83	0.254	0.291	0.044	-0.307	-0.242	-0.226			
84	0.145	0.085	0.003	-0.416	-0.448	-0.267			
85	0.070	0.115	0.000	-0.491	-0.418	-0.270			
86	0.140	0.178	0.118	-0.421	-0.355	-0.152			
87	0.071	0.222	0.020	-0.490	-0.311	-0.250			
88	0.122	0.081	0.061	-0.439	-0.452	-0.209			
89	0.062	0.173	0.058	-0.499	-0.360	-0.212			
90	0.050	0.197	0.011	-0.511	-0.336	-0.259			
91	0.051	0.271	0.019	-0.510	-0.262	-0.251			
92	0.139	0.215	0.041	-0.422	-0.318	-0.229			
93	0.038	0.063	0.000	-0.523	-0.470	-0.270			
94	0.035	0.165	0.004	-0.526	-0.368	-0.266			
95	0.016	0.132	0.061	-0.545	-0.401	-0.209			
96	0.135	0.148	0.045	-0.426	-0.385	-0.225			
97	0.041	0.112	0.000	-0.520	-0.421	-0.270			
98	0.137	0.218	0.023	-0.424	-0.315	-0.247			
99	0.021	0.122	0.000	-0.540	-0.411	-0.270			
100	0.063	0.094	0.013	-0.498	-0.439	-0.257			
101	0.143	0.203	0.094	-0.418	-0.330	-0.176			
102	0.126	0.251	0.025	-0.435	-0.282	-0.245			
103	0.000	0.073	0.003	-0.561	-0.460	-0.267			
104	0.095	0.185	-0.001	-0.466	-0.348	-0.271			
105	0.011	0.086	-0.001	-0.550	-0.447	-0.271			
106	0.073	0.192	0.009	-0.488	-0.341	-0.261			
107	0.140	0.208	0.027	-0.421	-0.325	-0.243			
108	0.131	0.169	0.109	-0.430	-0.364	-0.161			
109	0.165	0.120	0.129	-0.396	-0.413	-0.141			
110	0.135	0.142	0.007	-0.426	-0.391	-0.263			
111	0.074	0.096	0.030	-0.487	-0.437	-0.240			
112	0.243	0.189	0.312	-0.318	-0.344	0.042			+
113	0.100	0.234	0.036	-0.461	-0.299	-0.234			
114	0.055	0.166	0.041	-0.506	-0.367	-0.229			
115	0.828	0.144	0.123	0.267	-0.389	-0.147	+		
116	0.120	0.417	0.067	-0.441	-0.116	-0.203			
117	0.134	0.143	0.006	-0.427	-0.390	-0.264			
118	0.139	0.184	0.045	-0.422	-0.349	-0.225			
119	0.148	0.071	0.002	-0.413	-0.462	-0.268			
120	0.082	0.132	0.100	-0.479	-0.401	-0.170			
121	0.071	0.090	0.041	-0.490	-0.443	-0.229			
122	0.066	0.129	0.090	-0.495	-0.404	-0.180			
123	0.045	0.248	0.122	-0.516	-0.285	-0.148			
124	0.078	0.172	0.032	-0.483	-0.361	-0.238			
125	0.117	0.147	0.092	-0.444	-0.386	-0.178			
126	0.120	0.295	0.078	-0.441	-0.238	-0.192			
127	0.129	0.328	0.078	-0.432	-0.205	-0.192			
128	0.101	0.247	0.087	-0.460	-0.286	-0.183			
129	0.059	0.225	0.076	-0.502	-0.308	-0.194			
130	0.274	0.229	0.084	-0.287	-0.304	-0.186			
131	0.079	0.123	0.031	-0.482	-0.410	-0.239			
132	0.253	0.181	0.176	-0.308	-0.352	-0.094			
133	0.281	0.158	0.082	-0.280	-0.375	-0.188			
134	0.140	0.224	0.006	-0.421	-0.309	-0.264			
135	0.183	0.242	0.021	-0.378	-0.291	-0.249			
136	0.249	0.242	0.070	-0.312	-0.291	-0.200			
137	0.127	0.560	0.032	-0.434	0.027	-0.238		+	
138	0.113	0.257	0.028	-0.448	-0.276	-0.242			
139	0.143	0.237	0.121	-0.418	-0.296	-0.149			
140	0.117	0.123	0.032	-0.444	-0.410	-0.238			
141	0.150	0.303	0.117	-0.411	-0.230	-0.153			
142	0.193	0.212	0.030	-0.368	-0.321	-0.240			
143	0.293	0.381	0.121	-0.268	-0.152	-0.149			
144	0.394	0.166	0.033	-0.167	-0.367	-0.237			
145	0.233	0.141	0.022	-0.328	-0.392	-0.248			
146	0.016	0.086	0.001	-0.545	-0.447	-0.269			
147	0.154	0.118	0.007	-0.407	-0.415	-0.263			
148	0.092	0.216	0.080	-0.469	-0.317	-0.190			

	A	B	C	D	E	F	G	H	I
149	IgG	IgA	IgE	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
150	0.085	0.178	0.055	-0.476	-0.355	-0.215			
151	0.035	0.254	0.001	-0.526	-0.279	-0.269			
152	0.150	0.098	0.139	-0.411	-0.435	-0.131			
153	0.092	0.136	0.115	-0.469	-0.397	-0.155			
154	0.198	0.151	0.018	-0.363	-0.382	-0.252			
155	0.245	0.633	0.124	-0.316	0.100	-0.146		+	
156	0.082	0.186	0.121	-0.479	-0.347	-0.149			

	A	B	C	D	E	F	G	H
1	Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+
2	830	0.354	0.198	0.143	-0.207	-0.335	-0.127	
3	831	0.200	0.101	0.016	-0.361	-0.432	-0.254	
4	832	0.098	0.113	0.032	-0.463	-0.420	-0.238	
5	833	0.317	0.065	0.026	-0.244	-0.468	-0.244	
6	834	0.491	0.122	0.201	-0.070	-0.411	-0.069	
7	835	0.735	0.068	0.024	0.174	-0.465	-0.246	+
8	836	0.180	0.074	0.116	-0.381	-0.459	-0.154	
9	837	0.439	0.071	0.045	-0.122	-0.462	-0.225	
10	838	0.351	0.210	0.177	-0.210	-0.323	-0.093	
11	839	0.318	0.087	0.026	-0.243	-0.446	-0.244	
12	840	0.470	0.167	0.032	-0.091	-0.367	-0.238	
13	841	0.255	0.097	0.058	-0.306	-0.436	-0.212	
14	842	0.635	0.128	0.024	0.074	-0.405	-0.246	+
15	843	0.530	0.102	0.034	-0.031	-0.431	-0.236	
16	844	0.295	0.059	0.008	-0.266	-0.474	-0.262	
17	845	0.379	0.160	0.045	-0.182	-0.373	-0.225	
18	846	0.510	0.117	0.164	-0.051	-0.416	-0.106	
19	847	0.496	0.063	0.016	-0.065	-0.470	-0.254	
20	848	0.392	0.157	0.121	-0.169	-0.376	-0.149	
21	849	0.234	0.299	0.071	-0.327	-0.234	-0.199	
22	850	0.395	0.138	0.106	-0.166	-0.395	-0.164	
23	851	0.435	0.095	0.074	-0.126	-0.438	-0.196	
24	852	0.364	0.387	0.026	-0.197	-0.146	-0.244	
25	853	0.268	0.061	0.034	-0.293	-0.472	-0.236	
26	854	0.440	0.112	0.034	-0.121	-0.421	-0.236	
27	855	0.424	0.135	0.037	-0.137	-0.398	-0.233	
28	856	0.529	0.205	0.066	-0.032	-0.328	-0.204	
29	857	0.502	0.297	0.124	-0.059	-0.236	-0.146	
30	858	0.333	0.208	0.021	-0.228	-0.325	-0.249	
31	859	0.658	0.235	0.045	0.097	-0.298	-0.225	+
32	860	0.259	0.085	0.166	-0.302	-0.448	-0.104	
33	872	0.767	0.369	0.139	0.206	-0.164	-0.131	+

IgG	IgA	IgE	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+	No of E.g
0.600	0.651	0.229	0.039	0.118	-0.041	+	+		5000
0.873	1.538	0.417	0.312	1.005	0.147	+	+	+	24000
0.856	0.696	0.518	0.295	0.163	0.248	+	+	+	73000
0.534	0.293	0.400	-0.027	-0.240	0.130			+	2400
0.708	0.899	0.413	0.147	0.366	0.143	+	+	+	20600
1.704	1.282	0.460	1.143	0.749	0.190	+	+	+	66150
0.565	0.569	0.252	0.004	0.036	-0.018	+	+		302580
0.527	0.305	0.429	-0.034	-0.228	0.159			+	808
0.334	0.159	0.220	-0.227	-0.374	-0.050				7300
0.422	0.574	0.433	-0.139	0.041	0.163		+	+	300
0.540	0.742	0.308	-0.021	0.209	0.038		+	+	17600
0.253	0.156	0.244	-0.308	-0.377	-0.026				1400
0.968	1.005	0.450	0.407	0.472	0.180	+	+	+	18500
0.408	0.170	0.287	-0.153	-0.363	0.017			+	600
1.001	0.564	0.464	0.440	0.031	0.194	+	+	+	44000
1.086	0.322	0.568	0.525	-0.211	0.298	+		+	9000
0.797	0.746	0.277	0.236	0.213	0.007	+	+	+	9400
0.610	0.209	0.295	0.049	-0.324	0.025	+		+	9600
0.091	0.098	0.041	-0.470	-0.435	-0.229				2000
0.120	0.134	0.107	-0.441	-0.399	-0.163				5800
0.332	0.274	0.278	-0.229	-0.259	0.008			+	100080
1.155	1.036	0.423	0.594	0.503	0.153	+	+	+	17000
0.702	0.801	0.378	0.141	0.268	0.108	+	+	+	20
0.496	0.211	0.262	-0.065	-0.322	-0.008				80
0.198	0.164	0.228	-0.363	-0.369	-0.042				40
0.332	0.117	0.174	-0.229	-0.416	-0.096				1600
0.935	0.483	0.406	0.374	-0.050	0.136	+		+	6600
0.555	0.261	0.280	-0.006	-0.272	0.010			+	1000
0.557	0.255	0.243	-0.004	-0.278	-0.027				200
0.271	0.328	0.350	-0.290	-0.205	0.080			+	3
0.611	0.356	0.183	0.050	-0.177	-0.087	+			2000
0.322	0.357	0.049	-0.239	-0.176	-0.221				250
0.391	0.211	0.134	-0.170	-0.322	-0.136				1
0.317	0.290	0.255	-0.244	-0.243	-0.015				2700
0.768	0.486	0.522	0.207	-0.047	0.252	+		+	900
0.807	0.871	0.550	0.246	0.338	0.280	+	+	+	2000
0.613	0.120	0.216	0.052	-0.413	-0.054	+			60
0.996	1.004	0.440	0.435	0.471	0.170	+	+	+	200
0.562	0.670	0.264	0.001	0.137	-0.006	+	+		450
0.814	0.951	0.216	0.253	0.418	-0.054	+	+		4500
0.360	0.323	0.229	-0.201	-0.210	-0.041				800
0.552	0.350	0.267	-0.009	-0.183	-0.003				12120
0.300	0.235	0.148	-0.261	-0.298	-0.122				150
0.747	0.535	0.276	0.186	0.002	0.006	+	+	+	250
0.944	0.460	0.321	0.383	-0.073	0.051	+		+	1
0.966	0.392	0.360	0.405	-0.141	0.090	+		+	42000
0.618	0.382	0.199	0.057	-0.151	-0.071	+			500
0.979	1.543	0.151	0.418	1.010	-0.119	+	+		600
0.278	0.179	0.139	-0.283	-0.354	-0.131				2000
0.774	0.211	0.309	0.213	-0.322	0.039	+		+	3000
1.550	0.831	0.546	0.989	0.298	0.276	+	+	+	1200
1.104	0.539	0.497	0.543	0.006	0.227	+	+	+	31000
0.868	1.097	0.446	0.307	0.564	0.176	+	+	+	8500
1.136	1.412	0.323	0.575	0.879	0.053	+	+	+	300160
1.053	0.695	0.454	0.492	0.162	0.184	+	+	+	1300
0.110	0.170	0.118	-0.451	-0.363	-0.152				1
0.467	0.190	0.101	-0.094	-0.343	-0.169				2300
0.267	0.141	0.143	-0.294	-0.392	-0.127				2
1.273	1.585	0.545	0.712	1.052	0.275	+	+	+	1
1.654	0.461	0.428	1.093	-0.072	0.158	+		+	550
0.809	0.585	0.498	0.248	0.052	0.228	+	+	+	1500
0.887	0.495	0.420	0.326	-0.038	0.150	+		+	13000
1.202	0.755	0.461	0.641	0.222	0.191	+	+	+	10000
0.502	0.684	0.308	-0.059	0.151	0.038		+	+	6000
0.708	0.394	0.424	0.147	-0.139	0.154	+		+	10200
1.023	0.579	0.484	0.462	0.046	0.214	+	+	+	11600
0.334	0.415	0.354	-0.227	-0.118	0.084			+	1100
0.654	0.250	0.192	0.093	-0.283	-0.078	+			17500
1.055	0.886	0.269	0.494	0.353	-0.001	+	+		150
0.999	0.548	0.527	0.438	0.015	0.257	+	+	+	11000
1.944	0.877	0.547	1.383	0.344	0.277	+	+	+	70000
1.503	1.557	0.502	0.942	1.024	0.232	+	+	+	50200
1.179	1.071	0.462	0.618	0.538	0.192	+	+	+	52380
1.899	1.935	0.521	1.338	1.402	0.251	+	+	+	680

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
1	0.377	0.158	0.151	-0.184	-0.375	-0.119			
2	0.590	0.179	0.219	0.029	-0.354	-0.051	+		
3	0.418	0.313	0.049	-0.143	-0.220	-0.221			
4	0.278	0.437	0.181	-0.283	-0.096	-0.089			
5	0.302	0.309	0.186	-0.259	-0.224	-0.084			
6	0.337	0.102	0.146	-0.224	-0.431	-0.124			
7	0.133	0.070	0.208	-0.428	-0.463	-0.062			
8	0.243	0.137	0.084	-0.318	-0.396	-0.186			
9	0.281	0.075	0.281	-0.280	-0.458	0.011			+
10	0.347	0.097	0.230	-0.214	-0.436	-0.041			
11	0.368	0.090	0.273	-0.193	-0.443	0.003			+
12	0.406	0.160	0.138	-0.155	-0.373	-0.132			
13	0.408	0.164	0.167	-0.153	-0.369	-0.103			
14	0.166	0.152	0.251	-0.395	-0.381	-0.019			
15	0.218	0.139	0.257	-0.343	-0.394	-0.014			
16	0.522	0.329	0.162	-0.039	-0.204	-0.108			
17	0.474	0.393	0.184	-0.087	-0.140	-0.086			
18	0.301	0.148	0.262	-0.260	-0.385	-0.008			
19	0.260	0.095	0.097	-0.301	-0.438	-0.173			
20	0.314	0.515	0.232	-0.247	-0.018	-0.038			
21	0.232	0.139	0.116	-0.329	-0.394	-0.154			
22	0.398	0.196	0.270	-0.163	-0.337	0.000			
23	0.237	0.370	0.189	-0.324	-0.163	-0.081			
24	0.405	0.126	0.032	-0.156	-0.407	-0.238			
25	0.467	0.082	0.151	-0.094	-0.451	-0.119			
26	0.312	0.485	0.140	-0.249	-0.048	-0.130			
27	0.253	0.150	0.162	-0.308	-0.383	-0.108			
28	0.198	0.187	0.170	-0.363	-0.346	-0.100			
29	0.340	0.193	0.138	-0.221	-0.340	-0.132			
30	0.341	0.102	0.124	-0.220	-0.431	-0.146			
31	0.333	0.215	0.216	-0.228	-0.318	-0.054			
32	0.419	0.141	0.081	-0.142	-0.392	-0.189			
33	0.244	0.157	0.248	-0.317	-0.376	-0.022			
34	0.614	0.063	0.146	0.053	-0.470	-0.124	+		
35	0.244	0.098	0.200	-0.317	-0.435	-0.070			
36	0.242	0.220	0.211	-0.319	-0.313	-0.059			
37	0.279	0.102	0.119	-0.282	-0.431	-0.151			
38	0.330	0.074	0.030	-0.231	-0.459	-0.240			
39	0.365	0.264	0.113	-0.196	-0.269	-0.157			
40	0.159	0.158	0.054	-0.402	-0.375	-0.216			
41	0.510	1.077	0.086	-0.051	0.544	-0.184		+	
42	0.299	0.253	0.194	-0.262	-0.280	-0.076			
43	0.248	0.065	0.359	-0.313	-0.468	0.089			+
44	0.298	0.107	0.194	-0.263	-0.426	-0.076			
45	0.275	0.061	0.313	-0.286	-0.472	0.043			+
46	0.281	0.069	0.267	-0.280	-0.464	-0.003			
47	0.341	0.051	0.243	-0.220	-0.482	-0.027			
48	0.359	0.740	0.381	-0.202	0.207	0.111		+	+
49	0.345	0.302	0.146	-0.216	-0.231	-0.124			
50	0.266	0.197	0.108	-0.295	-0.336	-0.162			
51	0.366	0.275	0.216	-0.195	-0.258	-0.054			
52	0.670	0.173	0.219	0.109	-0.360	-0.051	+		
53	0.349	0.088	0.238	-0.212	-0.445	-0.032			
54	0.487	0.896	0.197	-0.074	0.363	-0.073		+	
55	0.243	0.342	0.086	-0.318	-0.191	-0.184			
56	0.132	0.252	0.165	-0.429	-0.281	-0.105			
57	0.226	0.293	0.189	-0.335	-0.240	-0.081			
58	0.379	0.290	0.050	-0.182	-0.243	-0.220			
59	0.415	0.360	0.239	-0.146	-0.173	-0.031			
60	0.268	0.293	0.264	-0.293	-0.240	-0.006			
61	0.192	0.477	0.077	-0.369	-0.056	-0.193			
62	0.283	0.208	0.089	-0.278	-0.325	-0.181			
63	0.135	0.224	0.111	-0.426	-0.309	-0.159			
64	0.205	0.055	0.192	-0.356	-0.478	-0.078			
65	0.122	0.078	0.049	-0.439	-0.455	-0.221			
66	0.297	0.116	0.265	-0.264	-0.417	-0.005			
67	0.196	0.089	0.070	-0.365	-0.444	-0.200			
68	0.154	0.182	0.224	-0.407	-0.351	-0.046			
69	0.293	0.321	0.202	-0.268	-0.212	-0.068			
70	0.192	0.293	0.006	-0.369	-0.240	-0.264			
71	0.264	0.096	0.149	-0.297	-0.437	-0.122			
72	0.152	0.181	0.065	-0.409	-0.352	-0.205			
73	0.200	0.052	0.186	-0.361	-0.481	-0.084			
74	0.367	0.346	0.248	-0.194	-0.187	-0.022			

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
75	0.271	0.069	0.130	-0.290	-0.464	-0.140			
76	0.435	0.108	0.143	-0.126	-0.425	-0.127			
77	0.296	0.324	0.112	-0.265	-0.209	-0.158			
78	0.261	0.070	0.162	-0.300	-0.463	-0.108			
79	0.345	0.215	0.197	-0.216	-0.318	-0.073			
80	0.312	0.132	0.213	-0.249	-0.401	-0.057			
81	0.237	0.249	0.270	-0.324	-0.284	0.000			
82	0.211	0.104	0.259	-0.350	-0.429	-0.011			
83	0.450	0.304	0.219	-0.111	-0.229	-0.051			
84	0.667	0.336	0.233	0.106	-0.197	-0.037	+		
85	0.211	0.083	0.151	-0.350	-0.450	-0.119			
86	0.401	0.151	0.257	-0.160	-0.382	-0.014			
87	0.159	0.409	0.273	-0.402	-0.124	0.003			+
88	0.223	0.083	0.132	-0.338	-0.450	-0.138			
89	0.199	0.367	0.151	-0.362	-0.166	-0.119			
90	0.157	0.092	0.221	-0.404	-0.441	-0.049			
91	0.228	0.182	0.167	-0.333	-0.351	-0.103			
92	0.132	0.039	0.167	-0.429	-0.494	-0.103			
93	0.679	0.058	0.203	0.118	-0.475	-0.068	+		
94	0.446	1.041	0.246	-0.115	0.508	-0.024		+	
95	0.427	0.083	0.194	-0.134	-0.450	-0.076			
96	0.360	0.119	0.041	-0.201	-0.414	-0.230			
97	0.412	0.116	0.176	-0.149	-0.417	-0.095			
98	0.242	0.112	0.197	-0.319	-0.421	-0.073			
99	0.229	0.210	0.086	-0.332	-0.323	-0.184			
100	0.359	0.040	0.186	-0.202	-0.493	-0.084			
101	0.435	0.159	0.289	-0.126	-0.374	0.019			+
102	0.255	0.084	0.008	-0.306	-0.449	-0.262			
103	0.221	0.154	0.016	-0.340	-0.379	-0.254			
104	0.089	0.043	0.073	-0.472	-0.490	-0.197			
105	0.223	0.084	0.238	-0.338	-0.449	-0.032			
106	0.188	0.196	0.127	-0.373	-0.337	-0.143			
107	0.312	0.821	0.092	-0.249	0.288	-0.178		+	
108	0.122	0.089	0.022	-0.439	-0.444	-0.248			
109	0.157	0.518	0.011	-0.404	-0.015	-0.259			
110	0.092	0.184	0.005	-0.469	-0.349	-0.265			
111	0.146	0.024	0.054	-0.415	-0.509	-0.216			
112	0.195	0.215	0.032	-0.366	-0.318	-0.238			
113	0.293	0.074	0.162	-0.268	-0.459	-0.108			
114	0.191	0.180	0.100	-0.370	-0.353	-0.170			
115	0.173	0.058	0.192	-0.388	-0.475	-0.078			
116	0.285	0.080	0.213	-0.276	-0.453	-0.057			
117	0.429	0.111	0.197	-0.132	-0.422	-0.073			
118	0.395	0.080	0.000	-0.166	-0.453	-0.270			
119	0.221	0.124	0.243	-0.340	-0.409	-0.027			
120	0.349	0.089	0.111	-0.212	-0.444	-0.159			
121	0.322	0.147	0.194	-0.239	-0.386	-0.076			
122	0.457	0.109	0.092	-0.104	-0.424	-0.178			
123	0.733	0.244	0.173	0.172	-0.289	-0.097	+		
124	0.787	0.603	0.292	0.226	0.070	0.022	+	+	+
125	0.571	0.373	0.108	0.010	-0.160	-0.162	+		
126	0.255	0.142	0.138	-0.306	-0.391	-0.132			
127	0.759	0.363	0.151	0.198	-0.170	-0.119	+		
128	0.611	0.316	0.200	0.050	-0.217	-0.070	+		
129	0.580	0.123	0.211	0.019	-0.410	-0.059	+		
131	0.729	0.286	0.189	0.168	-0.247	-0.081	+		
132	0.449	0.198	0.251	-0.112	-0.335	-0.019			
133	0.493	0.062	0.105	-0.068	-0.471	-0.165			
134	0.483	0.174	0.254	-0.078	-0.359	-0.016			
135	0.301	0.122	0.300	-0.260	-0.411	0.030			+
136	0.379	0.171	0.200	-0.182	-0.362	-0.070			
137	0.659	0.263	0.108	0.098	-0.270	-0.162	+		
137	0.187	0.367	0.165	-0.374	-0.166	-0.105			
138	0.515	0.266	0.095	-0.046	-0.267	-0.176			
139	0.250	0.127	0.016	-0.311	-0.406	-0.254			
140	0.363	0.105	0.030	-0.198	-0.428	-0.240			
141	0.361	0.099	0.232	-0.200	-0.434	-0.038			
142	0.468	0.146	0.227	-0.093	-0.387	-0.043			
143	0.420	0.181	0.057	-0.141	-0.352	-0.213			
144	0.680	0.225	0.294	0.119	-0.308	0.024	+		+
145	0.385	0.065	0.062	-0.176	-0.468	-0.208			
146	0.294	0.167	0.281	-0.267	-0.366	0.011			+
147	0.335	0.169	0.143	-0.226	-0.364	-0.127			
148	0.344	0.049	0.167	-0.217	-0.484	-0.103			

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
149	0.530	0.144	0.221	-0.031	-0.389	-0.049			
150	0.503	0.224	0.113	-0.058	-0.309	-0.157			
151	0.420	0.177	0.143	-0.141	-0.356	-0.127			
152	0.498	0.099	0.149	-0.063	-0.434	-0.122			
153	0.478	0.135	0.089	-0.083	-0.398	-0.181			
154	0.448	0.177	1.088	-0.113	-0.356	0.818			+
155	0.251	0.070	0.119	-0.310	-0.463	-0.151			
156	0.457	0.122	0.154	-0.104	-0.411	-0.116			
157	0.259	0.104	0.062	-0.302	-0.429	-0.208			
158	0.548	0.144	0.270	-0.013	-0.389	0.000			
159	0.641	0.336	0.173	0.080	-0.197	-0.097	+		
160	0.145	0.015	0.030	-0.416	-0.518	-0.240			
160	0.454	0.271	0.065	-0.107	-0.262	-0.205			
161	0.639	0.318	0.272	0.078	-0.215	0.002	+		+
162	0.569	0.211	0.108	0.008	-0.322	-0.162	+		
164	0.516	0.153	0.221	-0.045	-0.380	-0.049			
165	0.456	0.154	0.119	-0.105	-0.379	-0.151			
166	0.718	0.335	0.237	0.157	-0.198	-0.033	+		
167	0.433	0.297	0.111	-0.128	-0.236	-0.159			
168	0.259	0.107	0.046	-0.302	-0.426	-0.224			
169	0.452	0.284	0.178	-0.109	-0.249	-0.092			
170	0.001	0.320	0.246	-0.560	-0.213	-0.024			
171	0.336	0.235	0.257	-0.225	-0.298	-0.014			
172	0.267	0.128	0.146	-0.294	-0.405	-0.124			
173	0.617	0.190	0.321	0.056	-0.343	0.051	+		+
174	0.441	0.078	0.219	-0.120	-0.455	-0.051			
175	0.591	0.278	0.146	0.030	-0.255	-0.124	+		
176	0.332	0.116	0.254	-0.229	-0.417	-0.016			
177	0.476	0.136	0.043	-0.085	-0.397	-0.227			
178	0.504	0.073	0.016	-0.057	-0.460	-0.254			
179	0.268	0.073	0.086	-0.293	-0.460	-0.184			
180	0.498	0.171	0.073	-0.063	-0.362	-0.197			
181	0.537	0.135	0.170	-0.024	-0.398	-0.100			
183	0.163	0.191	0.256	-0.398	-0.342	-0.014			
184	0.198	0.053	0.194	-0.363	-0.480	-0.076			
185	0.471	0.097	0.211	-0.090	-0.436	-0.059			
186	0.459	0.116	0.124	-0.102	-0.417	-0.146			
187	0.615	0.103	0.254	0.054	-0.430	-0.016	+		
188	0.885	0.215	0.181	0.324	-0.318	-0.089	+		
189	0.654	0.181	0.157	0.093	-0.352	-0.113	+		
190	0.712	0.112	0.035	0.151	-0.421	-0.235	+		
191	0.329	0.382	0.143	-0.232	-0.151	-0.127			
192	0.379	0.080	0.124	-0.182	-0.453	-0.146			
193	0.622	0.153	0.047	0.061	-0.380	-0.223	+		
194	0.503	0.150	0.018	-0.058	-0.383	-0.252			
195	0.611	0.208	0.038	0.050	-0.325	-0.232	+		
196	0.262	0.119	0.000	-0.299	-0.414	-0.270			
197	0.458	0.013	0.000	-0.103	-0.520	-0.270			
198	0.259	0.186	0.000	-0.302	-0.347	-0.270			
199	0.244	0.108	0.056	-0.317	-0.425	-0.214			
200	0.284	0.140	0.062	-0.277	-0.393	-0.208			
201	0.365	0.119	0.209	-0.196	-0.414	-0.061			
202	0.177	0.128	0.000	-0.384	-0.405	-0.270			
203	0.360	0.140	0.209	-0.201	-0.393	-0.061			
204	0.175	0.041	0.041	-0.386	-0.492	-0.229			
205	0.349	0.248	0.231	-0.212	-0.285	-0.039			
206	0.435	0.237	0.170	-0.126	-0.296	-0.100			
207	0.596	0.432	0.106	0.035	-0.101	-0.164	+		
208	0.559	0.205	0.106	-0.002	-0.328	-0.164			
209	0.492	0.046	0.183	-0.069	-0.487	-0.087			
210	0.500	0.151	0.142	-0.061	-0.382	-0.128			
211	0.395	0.358	0.251	-0.166	-0.175	-0.019			
212	0.441	0.070	0.159	-0.120	-0.463	-0.111			
213	0.567	0.235	0.145	0.006	-0.298	-0.125	+		
214	0.337	0.051	0.106	-0.224	-0.482	-0.164			
215	0.004	0.267	0.089	-0.557	-0.266	-0.182			
216	0.450	0.150	0.271	-0.111	-0.383	0.001			+
217	0.312	0.064	0.062	-0.249	-0.469	-0.208			
218	0.349	0.196	0.307	-0.212	-0.337	0.037			+
219	0.208	0.415	0.035	-0.353	-0.118	-0.235			
220	0.290	0.167	0.050	-0.271	-0.366	-0.220			
221	0.662	0.249	0.133	0.101	-0.284	-0.137	+		
222	0.430	0.368	0.189	-0.131	-0.165	-0.081			
223	0.570	0.144	0.074	0.009	-0.389	-0.196	+		

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
224	0.159	0.146	0.053	-0.402	-0.387	-0.217			
225	0.170	0.129	0.124	-0.391	-0.404	-0.146			
226	0.378	0.496	0.271	-0.183	-0.037	0.001			+
227	0.286	0.071	0.142	-0.275	-0.462	-0.128			
228	0.158	0.181	0.030	-0.403	-0.352	-0.241			
229	0.224	0.181	0.209	-0.337	-0.352	-0.061			
230	0.225	0.398	0.097	-0.336	-0.135	-0.173			
231	0.086	0.267	0.080	-0.475	-0.266	-0.190			
232	0.140	0.339	0.189	-0.421	-0.194	-0.081			
233	0.214	0.119	0.136	-0.347	-0.414	-0.134			
234	0.221	0.072	0.209	-0.340	-0.461	-0.061			
235	0.348	0.846	0.260	-0.213	0.313	-0.010		+	
236	0.488	0.564	0.044	-0.073	0.031	-0.226		+	
237	0.464	0.334	0.301	-0.097	-0.199	0.031			+
238	0.254	0.391	0.091	-0.307	-0.142	-0.179			
239	0.208	0.074	0.094	-0.353	-0.459	-0.176			
240	0.239	0.151	0.077	-0.322	-0.382	-0.193			
241	0.242	0.152	0.180	-0.319	-0.381	-0.090			
242	0.204	0.053	0.224	-0.357	-0.480	-0.046			
243	0.183	0.175	0.248	-0.378	-0.358	-0.022			
244	0.221	0.060	0.304	-0.340	-0.473	0.034			+
245	0.257	0.055	0.150	-0.304	-0.478	-0.120			
246	0.348	0.355	0.233	-0.213	-0.178	-0.037			
247	0.215	0.351	0.162	-0.346	-0.182	-0.108			
248	0.397	0.394	0.204	-0.164	-0.139	-0.066			
249	0.194	0.097	0.159	-0.367	-0.436	-0.111			
250	0.243	0.188	0.201	-0.318	-0.345	-0.069			
251	0.155	0.045	0.298	-0.406	-0.488	0.028			+
252	0.272	0.079	0.233	-0.289	-0.454	-0.037			
253	0.175	0.075	0.174	-0.386	-0.458	-0.096			
254	0.238	0.551	0.189	-0.323	0.018	-0.081		+	
255	0.333	1.192	0.254	-0.228	0.659	-0.016		+	
256	0.212	0.124	0.221	-0.349	-0.409	-0.049			
257	0.189	0.253	0.150	-0.372	-0.280	-0.120			
258	0.183	0.295	0.192	-0.378	-0.238	-0.078			
259	0.200	0.261	0.159	-0.361	-0.272	-0.111			
260	0.157	0.383	0.257	-0.404	-0.150	-0.013			
261	0.136	0.049	0.195	-0.425	-0.484	-0.075			
262	0.368	0.119	0.257	-0.193	-0.414	-0.013			
263	0.243	0.087	0.100	-0.318	-0.446	-0.170			
264	0.217	0.173	0.171	-0.344	-0.360	-0.099			
265	0.347	0.850	0.000	-0.214	0.317	-0.270		+	
266	0.185	0.080	0.221	-0.376	-0.453	-0.049			
267	0.267	0.090	0.224	-0.294	-0.443	-0.046			
268	0.267	0.102	0.286	-0.294	-0.431	0.016			+
269	0.304	0.318	0.277	-0.257	-0.215	0.007			+
270	0.376	0.753	0.207	-0.185	0.220	-0.064		+	
271	0.259	0.128	0.015	-0.302	-0.405	-0.255			
272	0.228	0.206	0.248	-0.333	-0.327	-0.022			
273	0.135	0.176	0.139	-0.426	-0.357	-0.131			
274	0.180	0.097	0.295	-0.381	-0.436	0.025			+
275	0.174	0.051	0.239	-0.387	-0.482	-0.031			
276	0.234	0.057	0.274	-0.327	-0.476	0.004			+
277	0.243	0.055	0.165	-0.318	-0.478	-0.105			
278	0.006	0.017	0.000	-0.555	-0.516	-0.270			
279	0.005	0.006	0.000	-0.556	-0.527	-0.270			
280	0.005	0.012	0.000	-0.556	-0.521	-0.270			
281	0.003	0.005	0.000	-0.558	-0.528	-0.270			
282	0.004	0.005	0.000	-0.557	-0.528	-0.270			
283	0.006	0.007	0.000	-0.555	-0.526	-0.270			
284	0.008	0.009	0.000	-0.553	-0.524	-0.270			
285	0.005	0.049	0.012	-0.556	-0.484	-0.258			
286	0.002	0.009	0.014	-0.559	-0.524	-0.256			
287	0.003	0.000	0.012	-0.558	-0.533	-0.258			
288	0.565	0.292	0.226	0.004	-0.241	-0.044	+		
289	0.400	0.099	0.245	-0.161	-0.434	-0.025			
290	0.840	0.243	0.152	0.279	-0.290	-0.118	+		
291	0.301	0.048	0.122	-0.260	-0.485	-0.148			
292	0.384	0.209	0.215	-0.177	-0.324	-0.055			
293	0.323	0.087	0.229	-0.238	-0.446	-0.041			
294	0.396	0.219	0.243	-0.165	-0.314	-0.027			
295	0.496	0.133	0.236	-0.065	-0.400	-0.034			
296	0.578	0.297	0.152	0.017	-0.236	-0.118	+		
297	0.446	0.169	0.175	-0.115	-0.364	-0.095			

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
298	0.355	0.169	0.199	-0.206	-0.364	-0.071			
299	0.645	0.195	0.213	0.084	-0.338	-0.057	+		
300	0.382	0.190	0.152	-0.179	-0.343	-0.118			
301	0.641	0.218	0.222	0.080	-0.315	-0.048	+		
302	0.328	0.048	0.111	-0.233	-0.485	-0.159			
303	0.579	0.117	0.240	0.018	-0.416	-0.030	+		
304	0.706	0.106	0.277	0.145	-0.427	0.007	+		+
305	0.396	0.169	0.132	-0.165	-0.364	-0.138			
306	0.727	0.408	0.171	0.166	-0.125	-0.099	+		
307	0.441	0.234	0.282	-0.120	-0.299	0.012			+
308	0.562	1.430	0.012	0.001	0.897	-0.258	+	+	
309	0.217	0.077	0.111	-0.344	-0.456	-0.159			
310	0.240	0.061	0.157	-0.321	-0.472	-0.113			
311	0.600	0.196	0.172	0.039	-0.337	-0.098	+		
312	0.366	0.680	0.178	-0.195	0.147	-0.092		+	
313	0.735	0.139	0.300	0.174	-0.394	0.030	+		+
314	0.604	0.308	0.162	0.043	-0.225	-0.108	+		
315	0.504	0.272	0.210	-0.057	-0.261	-0.060			
316	0.307	0.284	0.152	-0.254	-0.249	-0.118			
317	0.370	0.110	0.178	-0.191	-0.423	-0.092			
318	0.528	0.132	0.213	-0.033	-0.401	-0.057			
319	0.346	0.232	0.252	-0.215	-0.301	-0.018			
320	0.436	0.836	0.247	-0.125	0.303	-0.023		+	
321	0.386	0.190	0.083	-0.175	-0.343	-0.187			
322	0.355	0.206	0.173	-0.206	-0.327	-0.097			
323	0.393	0.906	0.148	-0.168	0.373	-0.122		+	
324	0.434	0.162	0.240	-0.127	-0.371	-0.030			
325	0.257	0.087	0.092	-0.304	-0.446	-0.178			
326	0.472	0.181	0.213	-0.089	-0.352	-0.057			
327	0.346	0.236	0.046	-0.215	-0.297	-0.224			
329	0.565	1.105	0.245	0.004	0.572	-0.025	+	+	
330	0.379	0.172	0.039	-0.182	-0.361	-0.231			
331	0.484	0.215	0.205	-0.077	-0.318	-0.065			
332	0.566	0.209	0.125	0.005	-0.324	-0.145	+		
333	0.203	0.079	0.028	-0.358	-0.454	-0.242			
334	0.182	0.078	0.120	-0.379	-0.455	-0.150			
335	0.350	0.054	0.065	-0.211	-0.479	-0.205			
337	0.204	0.033	0.030	-0.357	-0.500	-0.240			
338	0.235	0.098	0.030	-0.326	-0.435	-0.240			
339	0.281	0.204	0.180	-0.280	-0.329	-0.090			
340	0.192	0.111	0.109	-0.369	-0.422	-0.161			
341	0.451	0.086	0.055	-0.110	-0.447	-0.215			
342	0.083	0.098	0.213	-0.478	-0.435	-0.057			
343	0.275	0.082	0.102	-0.286	-0.451	-0.168			
344	0.221	0.183	0.060	-0.340	-0.350	-0.210			
345	0.540	0.968	0.208	-0.021	0.435	-0.062		+	
346	0.330	0.107	0.203	-0.231	-0.426	-0.067			
347	0.138	0.098	0.016	-0.423	-0.435	-0.254			
348	0.288	0.168	0.238	-0.273	-0.365	-0.032			
349	0.344	0.066	0.273	-0.217	-0.467	0.003			+
350	0.140	0.066	0.164	-0.421	-0.467	-0.106			
351	0.226	0.165	0.236	-0.335	-0.368	-0.034			
352	0.129	0.230	0.129	-0.432	-0.303	-0.141			
353	0.236	0.313	0.194	-0.325	-0.220	-0.076			
354	0.275	0.107	0.132	-0.286	-0.426	-0.138			
355	0.368	0.215	0.171	-0.193	-0.318	-0.099			
356	0.227	0.098	0.109	-0.334	-0.435	-0.161			
357	0.289	0.350	0.085	-0.272	-0.183	-0.185			
358	0.298	0.289	0.238	-0.263	-0.244	-0.032			
359	0.254	0.272	0.194	-0.307	-0.261	-0.076			
360	0.136	0.124	0.085	-0.425	-0.409	-0.185			
361	0.259	0.236	0.016	-0.302	-0.297	-0.254			
362	0.201	0.393	0.254	-0.360	-0.140	-0.016			
363	0.241	0.277	0.284	-0.320	-0.256	0.014			+
364	0.172	0.224	0.157	-0.389	-0.309	-0.113			
365	0.202	0.059	0.053	-0.359	-0.474	-0.217			
366	0.219	0.112	0.185	-0.342	-0.421	-0.085			
367	0.172	0.120	0.224	-0.389	-0.413	-0.046			
368	0.073	0.025	0.081	-0.488	-0.508	-0.189			
369	0.332	0.610	0.122	-0.229	0.077	-0.148		+	
370	0.069	0.017	0.196	-0.492	-0.516	-0.074			
371	0.072	0.025	0.042	-0.489	-0.508	-0.228			
372	0.131	0.017	0.032	-0.430	-0.516	-0.238			
373	0.205	0.077	0.196	-0.356	-0.456	-0.074			

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
374	0.295	0.051	0.215	-0.266	-0.482	-0.055			
375	0.516	0.074	0.106	-0.045	-0.459	-0.164			
376	0.265	0.073	0.106	-0.296	-0.460	-0.164			
377	0.164	0.243	0.117	-0.397	-0.290	-0.153			
378	0.329	0.048	0.254	-0.232	-0.485	-0.016			
379	0.244	0.234	0.127	-0.317	-0.299	-0.143			
380	0.324	0.057	0.219	-0.237	-0.476	-0.051			
381	0.071	0.251	0.158	-0.490	-0.282	-0.112			
383	0.286	0.269	0.214	-0.275	-0.264	-0.056			
384	0.146	0.027	0.080	-0.415	-0.506	-0.190			
385	0.375	0.213	0.155	-0.186	-0.320	-0.115			
386	0.050	0.017	0.055	-0.511	-0.516	-0.215			
387	0.066	0.062	0.041	-0.495	-0.471	-0.229			
388	0.158	0.046	0.210	-0.403	-0.487	-0.060			
389	0.094	0.059	0.019	-0.467	-0.474	-0.251			
390	0.170	0.091	0.199	-0.391	-0.442	-0.071			
391	0.223	0.159	0.155	-0.338	-0.374	-0.115			
392	0.195	0.076	0.207	-0.366	-0.457	-0.063			
393	0.206	0.032	0.083	-0.355	-0.501	-0.187			
394	0.188	0.101	0.080	-0.373	-0.432	-0.190			
395	0.329	0.078	0.039	-0.232	-0.455	-0.231			
396	0.200	0.055	0.099	-0.361	-0.478	-0.171			
397	0.289	0.134	0.097	-0.272	-0.399	-0.173			
398	0.325	0.147	0.149	-0.236	-0.386	-0.121			
399	0.321	0.054	0.232	-0.240	-0.479	-0.038			
400	0.264	0.049	0.124	-0.297	-0.484	-0.146			
401	0.120	0.036	0.080	-0.441	-0.497	-0.190			
402	0.294	0.196	0.315	-0.267	-0.337	0.045			+
403	0.225	0.155	0.080	-0.336	-0.378	-0.190			
404	0.381	0.173	0.155	-0.180	-0.360	-0.115			
405	0.278	0.215	0.066	-0.283	-0.318	-0.204			
406	0.429	0.203	0.017	-0.132	-0.330	-0.253			
407	0.389	0.164	0.055	-0.172	-0.369	-0.215			
408	0.521	0.123	0.099	-0.040	-0.410	-0.171			
409	0.421	0.252	0.028	-0.140	-0.281	-0.242			
410	0.626	0.226	0.080	0.065	-0.307	-0.190	+		
411	0.444	0.206	0.196	-0.117	-0.327	-0.074			
412	0.318	0.268	0.210	-0.243	-0.265	-0.060			
413	0.342	0.233	0.072	-0.219	-0.300	-0.198			
414	0.307	0.057	0.069	-0.254	-0.476	-0.201			
415	0.738	0.122	0.166	0.177	-0.411	-0.104	+		
416	0.227	0.107	0.075	-0.334	-0.426	-0.195			
417	0.243	0.048	0.138	-0.318	-0.485	-0.132			
418	0.288	0.168	0.149	-0.273	-0.365	-0.121			
419	0.294	0.068	0.063	-0.267	-0.465	-0.207			
420	0.187	0.043	0.030	-0.374	-0.490	-0.240			
421	0.472	0.058	0.282	-0.089	-0.475	0.012			+
422	0.394	0.104	0.077	-0.167	-0.429	-0.193			
423	0.726	0.331	0.036	0.165	-0.202	-0.234	+		
424	0.206	0.034	0.168	-0.355	-0.499	-0.102			
425	0.325	0.034	0.190	-0.236	-0.499	-0.080			
426	0.384	0.105	0.259	-0.177	-0.428	-0.011			
427	0.246	0.150	0.210	-0.315	-0.383	-0.060			
428	0.405	0.075	0.069	-0.156	-0.458	-0.201			
429	0.490	0.114	0.182	-0.071	-0.419	-0.088			
430	0.400	0.133	0.149	-0.161	-0.400	-0.121			
431	0.860	0.293	0.089	0.299	-0.240	-0.181	+		
432	0.377	0.061	0.094	-0.184	-0.472	-0.176			
433	0.550	0.215	0.234	-0.011	-0.318	-0.036			
434	0.447	0.110	0.273	-0.114	-0.423	0.003			+
435	0.344	0.060	0.069	-0.217	-0.473	-0.201			
436	0.445	0.205	0.157	-0.116	-0.328	-0.113			
437	0.337	0.199	0.050	-0.224	-0.334	-0.220			
438	0.410	0.127	0.091	-0.151	-0.406	-0.179			
439	0.297	0.070	0.163	-0.264	-0.463	-0.107			
440	0.290	0.077	0.050	-0.271	-0.456	-0.220			
441	0.568	0.187	0.168	0.007	-0.346	-0.102	+		
442	0.405	0.090	0.221	-0.156	-0.443	-0.049			
443	0.196	0.019	0.179	-0.365	-0.514	-0.091			
444	0.666	0.108	0.066	0.105	-0.425	-0.204	+		
445	0.429	0.156	0.157	-0.132	-0.377	-0.113			
446	0.700	0.246	0.132	0.139	-0.287	-0.138	+		
447	0.390	0.204	0.190	-0.171	-0.329	-0.080			
448	0.405	0.259	0.044	-0.156	-0.274	-0.226			

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
449	0.376	0.123	0.179	-0.185	-0.410	-0.091			
450	0.530	0.165	0.171	-0.031	-0.368	-0.099			
451	0.369	0.066	0.199	-0.192	-0.467	-0.071			
452	0.599	0.150	0.235	0.038	-0.383	-0.035	+		
453	0.245	0.081	0.226	-0.316	-0.452	-0.044			
454	0.474	0.138	0.061	-0.087	-0.395	-0.209			
455	0.325	0.099	0.226	-0.236	-0.434	-0.044			
457	0.391	0.132	0.144	-0.170	-0.401	-0.126			
456	0.185	0.030	0.293	-0.376	-0.503	0.023			+
458	0.438	0.269	0.072	-0.123	-0.264	-0.198			
459	0.395	0.059	0.315	-0.166	-0.474	0.045			+
460	0.394	0.269	0.271	-0.167	-0.264	0.001			+
461	0.611	0.342	0.098	0.050	-0.191	-0.172	+		
462	0.573	0.098	0.276	0.012	-0.435	0.006	+		+
464	0.405	0.138	0.265	-0.156	-0.395	-0.005			
465	0.423	0.198	0.331	-0.138	-0.335	0.061			+
466	0.561	0.498	0.210	0.000	-0.035	-0.060			
468	0.484	0.052	0.235	-0.077	-0.481	-0.035			
469	0.288	0.076	0.240	-0.273	-0.457	-0.030			
470	0.266	0.037	0.144	-0.295	-0.496	-0.126			
471	0.288	0.125	0.204	-0.273	-0.408	-0.066			
472	0.362	0.356	0.207	-0.199	-0.177	-0.063			
473	0.383	0.125	0.337	-0.178	-0.408	0.067			+
474	0.318	0.078	0.276	-0.243	-0.455	0.006			+
475	0.193	0.067	0.135	-0.368	-0.466	-0.135			
476	0.564	0.085	0.224	0.003	-0.448	-0.046	+		
477	0.186	0.485	0.131	-0.375	-0.048	-0.139			
478	0.631	0.135	0.290	0.070	-0.398	0.020	+		+
479	0.547	0.217	0.273	-0.014	-0.316	0.003			+
480	0.557	0.103	0.298	-0.004	-0.430	0.028			+
481	0.648	0.423	0.254	0.087	-0.110	-0.016	+		
482	0.633	0.152	0.235	0.072	-0.381	-0.035	+		
483	0.444	0.307	0.026	-0.117	-0.226	-0.244			
484	0.639	0.085	0.086	0.078	-0.448	-0.184	+		
485	0.427	0.170	0.310	-0.134	-0.363	0.040			+
486	0.364	0.303	0.306	-0.197	-0.230	0.036			+
487	0.600	0.333	0.384	0.039	-0.200	0.114	+		+
488	0.602	0.414	0.123	0.041	-0.119	-0.147	+		
489	0.603	0.258	0.298	0.042	-0.275	0.028	+		+
490	0.461	0.126	0.119	-0.100	-0.407	-0.151			
491	0.617	0.309	0.048	0.056	-0.224	-0.222	+		
492	0.346	0.297	0.151	-0.215	-0.236	-0.119			
493	0.343	0.212	0.328	-0.218	-0.321	0.058			+
494	0.533	0.368	0.369	-0.028	-0.165	0.099			+
495	0.192	0.249	0.153	-0.369	-0.284	-0.117			
496	0.317	0.269	0.060	-0.244	-0.264	-0.210			
497	0.514	0.098	0.410	-0.047	-0.435	0.140			+
498	0.602	0.155	0.235	0.041	-0.378	-0.035	+		
499	0.501	0.047	0.108	-0.060	-0.486	-0.162			
500	0.527	0.294	0.035	-0.034	-0.239	-0.235			
501	0.396	0.070	0.261	-0.165	-0.463	-0.009			
502	0.398	0.094	0.030	-0.163	-0.439	-0.240			
503	0.325	0.084	0.078	-0.236	-0.449	-0.192			
504	0.357	0.028	0.127	-0.204	-0.505	-0.143			
505	0.578	0.068	0.205	0.017	-0.465	-0.065	+		
506	0.639	0.105	0.332	0.078	-0.428	0.062	+		+
507	1.096	0.451	0.280	0.535	-0.082	0.010	+		+
508	0.409	0.207	0.188	-0.152	-0.326	-0.082			
509	0.338	0.109	0.269	-0.223	-0.424	-0.001			
510	1.199	0.963	0.063	0.638	0.430	-0.207	+	+	
511	0.376	0.154	0.078	-0.185	-0.379	-0.192			
512	0.357	0.070	0.116	-0.204	-0.463	-0.154			
513	0.911	0.119	0.131	0.350	-0.414	-0.139	+		
514	0.277	0.452	0.433	-0.284	-0.081	0.163			+
515	0.323	0.151	0.388	-0.238	-0.382	0.118			+
516	0.471	0.112	0.328	-0.090	-0.421	0.058			+
517	0.802	0.524	0.116	0.241	-0.009	-0.154	+		
518	0.833	0.628	0.060	0.272	0.095	-0.210	+	+	
519	0.890	0.219	0.270	0.329	-0.314	0.000	+		
520	0.484	0.084	0.190	-0.077	-0.449	-0.080			
521	0.802	0.139	0.056	0.241	-0.394	-0.214	+		
522	0.511	0.074	0.190	-0.050	-0.459	-0.080			
523	0.441	0.086	0.246	-0.120	-0.447	-0.024			
524	0.473	0.238	0.448	-0.088	-0.295	0.178			+

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
525	0.423	0.152	0.034	-0.138	-0.381	-0.236			
526	0.562	0.467	0.266	0.001	-0.066	-0.004	+		
527	0.503	0.164	0.104	-0.058	-0.369	-0.166			
528	0.365	0.271	0.235	-0.196	-0.262	-0.035			
529	0.207	0.098	0.362	-0.354	-0.435	0.092			+
530	0.723	0.466	0.119	0.162	-0.067	-0.151	+		
531	0.455	0.181	0.250	-0.106	-0.352	-0.020			
532	0.563	0.098	0.041	0.002	-0.435	-0.229	+		
533	0.784	0.226	0.186	0.223	-0.307	-0.084	+		
534	0.534	0.101	0.060	-0.027	-0.432	-0.210			
535	0.458	0.137	0.108	-0.103	-0.396	-0.162			
536	0.250	0.075	0.131	-0.311	-0.458	-0.139			
537	0.691	0.096	0.246	0.130	-0.437	-0.024	+		
538	0.815	0.003	0.201	0.254	-0.530	-0.069	+		
539	0.287	0.121	0.500	-0.274	-0.412	0.230			+
540	0.208	0.036	0.164	-0.353	-0.497	-0.106			
541	0.387	0.082	0.317	-0.174	-0.451	0.047			+
542	0.519	0.178	0.336	-0.042	-0.355	0.066			+
543	0.418	0.114	0.082	-0.143	-0.419	-0.188			
544	0.367	0.043	0.298	-0.194	-0.490	0.028			+
545	0.657	0.128	0.317	0.096	-0.405	0.047	+		+
546	0.225	0.083	0.242	-0.336	-0.450	-0.028			
547	0.361	0.088	0.131	-0.200	-0.445	-0.139			
548	0.690	1.524	0.231	0.129	0.991	-0.039	+	+	
549	0.516	0.222	0.142	-0.045	-0.311	-0.128			
550	0.195	0.071	0.160	-0.366	-0.462	-0.110			
551	0.278	0.103	0.257	-0.283	-0.430	-0.013			
552	0.273	0.071	0.198	-0.288	-0.462	-0.072			
553	0.301	0.053	0.231	-0.260	-0.480	-0.039			
554	0.444	0.081	0.075	-0.117	-0.452	-0.195			
555	0.428	0.090	0.242	-0.133	-0.443	-0.028			
556	0.413	0.202	0.302	-0.148	-0.331	0.032			+
557	0.373	0.138	0.332	-0.188	-0.395	0.062			+
558	0.411	0.259	0.113	-0.150	-0.274	-0.157			
559	0.687	0.334	0.157	0.126	-0.199	-0.113	+		
560	0.642	0.159	0.265	0.081	-0.374	-0.005	+		
561	0.295	0.351	0.228	-0.266	-0.182	-0.042			
562	0.554	0.178	0.045	-0.007	-0.355	-0.225			
563	0.216	0.027	0.157	-0.345	-0.506	-0.113			
564	0.351	0.086	0.317	-0.210	-0.447	0.047			+
565	0.266	0.068	0.489	-0.295	-0.465	0.219			+
567	0.668	0.094	0.242	0.107	-0.439	-0.028	+		
568	0.458	0.070	0.373	-0.103	-0.463	0.103			+
569	0.378	0.102	0.332	-0.183	-0.431	0.062			+
570	0.556	0.096	0.388	-0.005	-0.437	0.118			+
571	0.273	0.079	0.444	-0.288	-0.454	0.174			+
572	0.283	0.096	0.149	-0.278	-0.437	-0.121			
573	0.410	0.095	0.093	-0.151	-0.438	-0.177			
574	0.360	0.160	0.213	-0.201	-0.373	-0.057			
575	0.354	0.085	0.418	-0.207	-0.448	0.148			+
576	0.387	0.059	0.347	-0.174	-0.474	0.077			+
577	0.329	0.246	0.176	-0.232	-0.287	-0.094			
578	0.325	0.102	0.121	-0.236	-0.431	-0.149			
579	0.268	0.095	0.195	-0.293	-0.438	-0.075			
580	0.268	0.436	0.110	-0.293	-0.097	-0.160			
581	0.570	0.204	0.216	0.009	-0.329	-0.054	+		
582	0.185	0.028	0.103	-0.376	-0.505	-0.167			
583	0.175	0.219	0.210	-0.386	-0.314	-0.060			
584	0.280	0.072	0.074	-0.281	-0.461	-0.196			
585	0.236	0.270	0.147	-0.325	-0.263	-0.123			
586	0.276	0.216	0.048	-0.285	-0.317	-0.222			
587	0.749	0.296	0.253	0.188	-0.237	-0.017	+		
588	0.111	0.088	0.026	-0.450	-0.445	-0.244			
589	0.195	0.103	0.061	-0.366	-0.430	-0.209			
590	0.375	0.189	0.137	-0.186	-0.344	-0.133			
591	0.416	0.534	0.108	-0.145	0.001	-0.162		+	
592	0.592	0.198	0.098	0.031	-0.335	-0.172	+		
593	0.054	0.015	0.092	-0.507	-0.518	-0.178			
594	0.038	0.032	0.029	-0.523	-0.501	-0.241			
595	0.053	0.021	0.011	-0.508	-0.512	-0.259			
596	0.206	0.200	0.119	-0.355	-0.333	-0.151			
597	0.052	0.023	0.161	-0.509	-0.510	-0.109			
598	0.111	0.033	0.058	-0.450	-0.500	-0.212			
599	0.149	0.085	0.396	-0.412	-0.448	0.126			+

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
600	0.118	0.054	0.029	-0.443	-0.479	-0.241			
601	0.035	0.030	0.024	-0.526	-0.503	-0.246			
602	0.128	0.039	0.195	-0.433	-0.494	-0.075			
603	0.066	0.022	0.018	-0.495	-0.511	-0.252			
604	0.391	0.211	0.215	-0.170	-0.322	-0.055			
605	0.094	0.116	0.079	-0.467	-0.417	-0.191			
606	0.075	0.026	0.032	-0.486	-0.507	-0.238			
607	0.547	0.093	0.158	-0.014	-0.440	-0.112			
608	0.304	0.152	0.090	-0.257	-0.381	-0.180			
609	0.418	0.318	0.084	-0.143	-0.215	-0.186			
610	0.586	0.207	0.050	0.025	-0.326	-0.220	+		
611	0.679	0.085	0.195	0.118	-0.448	-0.075	+		
612	0.428	0.113	0.190	-0.133	-0.420	-0.080			
613	0.306	0.035	0.230	-0.255	-0.498	-0.040			
614	0.635	0.117	0.048	0.074	-0.416	-0.222	+		
615	0.779	0.178	0.095	0.218	-0.355	-0.175	+		
616	0.336	0.364	0.121	-0.225	-0.169	-0.149			
617	0.377	0.055	0.161	-0.184	-0.478	-0.109			
618	0.163	0.032	0.106	-0.398	-0.501	-0.164			
619	0.472	0.286	0.231	-0.089	-0.247	-0.039			
620	0.462	0.069	0.177	-0.099	-0.464	-0.093			
621	0.504	0.192	0.040	-0.057	-0.341	-0.230			
622	0.207	0.090	0.071	-0.354	-0.443	-0.199			
623	0.300	0.192	0.137	-0.261	-0.341	-0.133			
624	0.282	0.041	0.243	-0.279	-0.492	-0.027			
625	0.530	0.089	0.166	-0.031	-0.444	-0.104			
626	0.462	0.067	0.180	-0.099	-0.466	-0.090			
627	0.267	0.084	0.150	-0.294	-0.449	-0.120			
628	0.896	0.800	0.251	0.335	0.267	-0.019	+	+	
629	0.266	0.144	0.166	-0.295	-0.389	-0.104			
630	0.395	0.096	0.077	-0.166	-0.437	-0.193			
631	0.203	0.569	0.090	-0.358	0.036	-0.180		+	
632	0.542	0.123	0.264	-0.019	-0.410	-0.006			
633	0.423	0.083	0.135	-0.138	-0.450	-0.135			
634	0.340	0.074	0.219	-0.221	-0.459	-0.051			
635	0.639	0.204	0.079	0.078	-0.329	-0.191	+		
636	0.348	0.118	0.045	-0.213	-0.415	-0.225			
637	0.489	0.280	0.184	-0.072	-0.253	-0.086			
638	0.534	0.089	0.150	-0.027	-0.444	-0.120			
639	0.318	0.211	0.206	-0.243	-0.322	-0.064			
640	1.010	0.594	0.354	0.449	0.061	0.084	+	+	+
641	0.291	0.139	0.219	-0.270	-0.394	-0.051			
642	0.449	0.083	0.124	-0.112	-0.450	-0.146			
643	0.286	0.058	0.087	-0.275	-0.475	-0.183			
644	0.571	0.167	0.166	0.010	-0.366	-0.104	+		
645	0.305	0.068	0.108	-0.256	-0.465	-0.162			
646	0.389	0.115	0.048	-0.172	-0.418	-0.222			
647	0.311	0.060	0.116	-0.250	-0.473	-0.154			
648	0.189	0.081	0.048	-0.372	-0.452	-0.222			
649	0.542	0.110	0.100	-0.019	-0.423	-0.170			
650	0.296	0.141	0.232	-0.265	-0.392	-0.038			
651	0.304	0.118	0.124	-0.257	-0.415	-0.146			
652	0.329	0.071	0.114	-0.232	-0.462	-0.156			
653	0.256	0.065	0.061	-0.305	-0.468	-0.209			
654	0.318	0.149	0.158	-0.243	-0.384	-0.112			
655	0.264	0.148	0.084	-0.297	-0.385	-0.186			
658	0.312	0.249	0.280	-0.249	-0.284	0.010			+
656	0.249	0.390	0.275	-0.312	-0.143	0.005			+
657	0.398	0.247	0.193	-0.163	-0.286	-0.077			
659	0.234	0.157	0.053	-0.327	-0.376	-0.217			
660	0.140	0.275	0.063	-0.421	-0.258	-0.207			
661	0.229	0.339	0.082	-0.332	-0.194	-0.188			
662	0.188	0.063	0.280	-0.373	-0.470	0.010			+
663	0.849	0.164	0.214	0.288	-0.369	-0.056	+		
664	0.217	0.420	0.264	-0.344	-0.113	-0.006			
665	0.150	0.083	0.100	-0.411	-0.450	-0.170			
666	0.003	0.246	0.246	-0.558	-0.287	-0.024			
667	0.324	0.579	0.219	-0.237	0.046	-0.051		+	
668	0.354	0.086	0.238	-0.207	-0.447	-0.032			
669	0.225	0.082	0.135	-0.336	-0.451	-0.135			
670	0.225	0.067	0.214	-0.336	-0.466	-0.056			
671	0.193	0.092	0.121	-0.368	-0.441	-0.149			
672	0.209	0.097	0.150	-0.352	-0.436	-0.120			
673	0.208	0.031	0.166	-0.353	-0.502	-0.104			

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
674	0.531	0.076	0.150	-0.030	-0.457	-0.120			
675	0.135	0.065	0.264	-0.426	-0.468	-0.006			
676	0.769	0.298	0.285	0.208	-0.235	0.015	+		+
677	0.361	0.235	0.122	-0.200	-0.298	-0.148			
678	0.416	0.334	0.229	-0.145	-0.199	-0.041			
679	0.626	0.546	0.066	0.065	0.013	-0.204	+	+	
680	0.232	0.142	0.036	-0.329	-0.391	-0.234			
681	0.259	0.264	0.205	-0.302	-0.269	-0.065			
682	0.453	0.185	0.177	-0.108	-0.348	-0.093			
683	0.220	0.112	0.122	-0.341	-0.421	-0.148			
684	0.209	0.294	0.011	-0.352	-0.239	-0.259			
685	0.300	0.232	0.058	-0.261	-0.301	-0.212			
686	0.311	0.091	0.136	-0.250	-0.442	-0.134			
687	0.463	0.077	0.111	-0.098	-0.456	-0.159			
688	0.463	0.330	0.011	-0.098	-0.203	-0.259			
689	0.274	0.092	0.111	-0.287	-0.441	-0.159			
690	0.408	0.051	0.127	-0.153	-0.482	-0.143			
691	0.283	0.195	0.061	-0.278	-0.338	-0.209			
692	0.459	0.220	0.055	-0.102	-0.313	-0.215			
693	0.292	0.285	0.199	-0.269	-0.248	-0.071			
694	0.204	0.052	0.064	-0.357	-0.481	-0.206			
695	0.264	0.258	0.236	-0.297	-0.275	-0.034			
696	0.682	0.439	0.171	0.121	-0.094	-0.099	+		
697	0.199	0.098	0.069	-0.362	-0.435	-0.201			
698	0.661	0.166	0.075	0.100	-0.367	-0.195	+		
699	0.357	0.235	0.204	-0.204	-0.298	-0.066			
700	0.311	0.045	0.097	-0.250	-0.488	-0.173			
701	0.377	0.091	0.072	-0.184	-0.442	-0.198			
702	0.259	0.168	0.188	-0.302	-0.365	-0.082			
703	0.328	0.085	0.188	-0.233	-0.448	-0.082			
704	0.217	0.257	0.044	-0.344	-0.276	-0.226			
705	0.319	0.055	0.080	-0.242	-0.478	-0.190			
706	0.251	0.145	0.108	-0.310	-0.388	-0.162			
707	0.255	0.064	0.152	-0.306	-0.469	-0.118			
708	0.563	0.187	0.114	0.002	-0.346	-0.156	+		
709	0.546	0.241	0.116	-0.015	-0.292	-0.154			
710	0.425	0.150	0.100	-0.136	-0.383	-0.170			
711	0.356	0.082	0.072	-0.205	-0.451	-0.198			
712	0.255	0.168	0.094	-0.306	-0.365	-0.176			
713	0.359	0.069	0.083	-0.202	-0.464	-0.187			
714	0.344	0.074	0.111	-0.217	-0.459	-0.159			
715	0.415	0.060	0.147	-0.146	-0.473	-0.123			
716	0.447	0.099	0.083	-0.114	-0.434	-0.187			
722	0.565	0.106	0.055	0.004	-0.427	-0.215	+		
719	0.344	0.083	0.122	-0.217	-0.450	-0.148			
718	0.251	0.080	0.122	-0.310	-0.453	-0.148			
720	0.360	0.190	0.080	-0.201	-0.343	-0.190			
721	0.235	0.033	0.050	-0.326	-0.500	-0.220			
717	0.199	0.055	0.186	-0.362	-0.478	-0.084			
723	0.433	0.056	0.161	-0.128	-0.477	-0.109			
724	0.302	0.133	0.161	-0.259	-0.400	-0.109			
725	0.246	0.049	0.130	-0.315	-0.484	-0.140			
726	0.269	0.096	0.130	-0.292	-0.437	-0.140			
727	0.163	0.093	0.183	-0.398	-0.440	-0.087			
728	0.273	0.226	0.100	-0.288	-0.307	-0.170			
729	0.310	0.099	0.075	-0.251	-0.434	-0.195			
730	0.203	0.108	0.075	-0.358	-0.425	-0.195			
731	0.114	0.038	0.039	-0.447	-0.495	-0.231			
732	0.332	0.128	0.150	-0.229	-0.405	-0.120			
733	0.217	0.056	0.177	-0.344	-0.477	-0.093			
734	0.283	0.129	0.100	-0.278	-0.404	-0.170			
735	0.340	0.135	0.158	-0.221	-0.398	-0.112			
736	0.237	0.038	0.130	-0.324	-0.495	-0.140			
737	0.265	0.064	0.083	-0.296	-0.469	-0.187			
738	0.370	0.060	0.053	-0.191	-0.473	-0.217			
739	0.414	0.047	0.061	-0.147	-0.486	-0.209			
740	0.653	0.159	0.055	0.092	-0.374	-0.215	+		
741	0.474	0.142	0.039	-0.087	-0.391	-0.231			
742	0.304	0.037	0.039	-0.257	-0.496	-0.231			
743	0.542	0.070	0.127	-0.019	-0.463	-0.143			
744	0.298	0.068	0.108	-0.263	-0.465	-0.162			
745	0.650	0.193	0.105	0.089	-0.340	-0.165	+		
746	0.465	0.021	0.208	-0.096	-0.512	-0.062			
747	0.335	0.086	0.197	-0.226	-0.447	-0.073			

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
748	0.395	0.080	0.072	-0.166	-0.453	-0.198			
749	0.301	0.588	0.190	-0.260	0.055	-0.080		+	
750	0.554	0.053	0.230	-0.007	-0.480	-0.040			
752	0.290	0.138	0.114	-0.271	-0.395	-0.156			
753	0.210	0.026	0.116	-0.351	-0.507	-0.154			
754	0.266	0.007	0.105	-0.295	-0.526	-0.165			
755	0.194	0.029	0.039	-0.367	-0.504	-0.231			
756	0.060	0.022	0.094	-0.501	-0.511	-0.176			
757	0.314	0.053	0.199	-0.247	-0.480	-0.071			
758	0.070	0.046	0.102	-0.491	-0.487	-0.168			
759	0.230	0.077	0.111	-0.331	-0.456	-0.159			
760	0.421	0.029	0.097	-0.140	-0.504	-0.173			
762	0.500	0.329	0.276	-0.061	-0.204	0.006			+
763	0.169	0.055	0.172	-0.392	-0.478	-0.098			
764	0.520	0.131	0.061	-0.041	-0.402	-0.209			
765	0.303	0.095	0.150	-0.258	-0.438	-0.120			
766	0.350	0.107	0.158	-0.211	-0.426	-0.112			
767	0.480	0.328	0.166	-0.081	-0.205	-0.104			
768	0.465	0.085	0.205	-0.096	-0.448	-0.065			
769	0.303	0.103	0.141	-0.258	-0.430	-0.129			
770	0.333	0.119	0.127	-0.228	-0.414	-0.143			
771	0.421	0.090	0.091	-0.140	-0.443	-0.179			
772	0.492	0.103	0.075	-0.069	-0.430	-0.195			
773	0.385	0.138	0.097	-0.176	-0.395	-0.173			
774	0.878	0.404	0.105	0.317	-0.129	-0.165	+		
775	0.321	0.079	0.119	-0.240	-0.454	-0.151			
776	0.211	0.056	0.100	-0.350	-0.478	-0.170			
777	0.346	0.128	0.214	-0.215	-0.405	-0.056			
778	0.381	0.313	0.249	-0.180	-0.220	-0.021			
779	0.822	0.305	0.042	0.261	-0.228	-0.228	+		
780	0.433	0.127	0.050	-0.128	-0.406	-0.220			
781	0.259	0.123	0.148	-0.302	-0.410	-0.122			
782	0.392	0.068	0.182	-0.169	-0.465	-0.088			
784	0.245	0.237	0.124	-0.316	-0.296	-0.146			
785	0.411	0.223	0.114	-0.150	-0.310	-0.156			
786	0.276	0.047	0.201	-0.285	-0.486	-0.069			
787	0.451	0.131	0.206	-0.110	-0.402	-0.064			
788	0.337	0.125	0.230	-0.224	-0.408	-0.040			
789	0.434	0.138	0.209	-0.127	-0.395	-0.061			
790	0.426	0.443	0.219	-0.135	-0.090	-0.051			
791	0.445	0.080	0.032	-0.116	-0.453	-0.238			
792	0.386	0.203	0.024	-0.175	-0.330	-0.246			
793	0.307	0.105	0.029	-0.254	-0.428	-0.241			
794	0.624	0.488	0.058	0.063	-0.045	-0.212	+		
795	0.264	0.270	0.199	-0.297	-0.263	-0.071			
796	0.482	0.081	0.016	-0.079	-0.452	-0.254			
797	0.542	0.314	0.095	-0.019	-0.219	-0.175			
798	0.312	0.263	0.079	-0.249	-0.270	-0.191			
799	0.347	0.164	0.045	-0.214	-0.369	-0.225			
800	0.293	0.102	0.021	-0.268	-0.431	-0.249			
801	0.522	0.071	0.034	-0.039	-0.462	-0.236			
802	0.532	0.122	0.040	-0.029	-0.411	-0.230			
803	0.283	0.138	0.084	-0.278	-0.395	-0.186			
804	0.437	0.114	0.116	-0.124	-0.419	-0.154			
805	0.240	0.216	0.209	-0.321	-0.317	-0.061			
806	0.475	0.105	0.042	-0.086	-0.428	-0.228			
807	0.312	0.046	0.061	-0.249	-0.487	-0.209			
808	0.467	0.062	0.106	-0.094	-0.471	-0.164			
809	0.317	0.024	0.145	-0.244	-0.509	-0.125			
810	0.306	0.104	0.050	-0.255	-0.429	-0.220			
811	0.621	0.103	0.127	0.060	-0.430	-0.143	+		
812	0.566	0.120	0.143	0.005	-0.413	-0.127	+		
813	0.456	0.040	0.048	-0.105	-0.493	-0.222			
814	0.260	0.039	0.026	-0.301	-0.494	-0.244			
815	0.383	0.113	0.090	-0.178	-0.420	-0.180			
816	0.335	0.245	0.172	-0.226	-0.288	-0.098			
817	0.369	0.344	0.045	-0.192	-0.189	-0.225			
818	0.266	0.304	0.205	-0.295	-0.229	-0.065			
819	0.262	0.065	0.135	-0.299	-0.468	-0.135			
820	0.352	0.221	0.107	-0.209	-0.312	-0.163			
821	0.344	0.105	0.135	-0.217	-0.428	-0.135			
822	0.432	0.054	0.153	-0.129	-0.479	-0.117			
823	0.362	0.089	0.084	-0.199	-0.444	-0.186			
824	0.442	0.163	0.127	-0.119	-0.370	-0.143			

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
825	0.529	0.144	0.058	-0.032	-0.389	-0.212			
826	0.381	0.154	0.100	-0.180	-0.379	-0.170			
827	0.303	0.098	0.008	-0.258	-0.435	-0.262			
828	0.507	0.176	0.048	-0.054	-0.357	-0.222			
829	0.391	0.278	0.229	-0.170	-0.255	-0.041			
830	0.354	0.198	0.143	-0.207	-0.335	-0.127			
831	0.200	0.101	0.016	-0.361	-0.432	-0.254			
832	0.098	0.113	0.032	-0.463	-0.420	-0.238			
833	0.317	0.065	0.026	-0.244	-0.468	-0.244			
834	0.491	0.122	0.201	-0.070	-0.411	-0.069			
835	0.735	0.068	0.024	0.174	-0.465	-0.246	+		
836	0.180	0.074	0.116	-0.381	-0.459	-0.154			
837	0.439	0.071	0.045	-0.122	-0.462	-0.225			
838	0.351	0.210	0.177	-0.210	-0.323	-0.093			
839	0.318	0.087	0.026	-0.243	-0.446	-0.244			
840	0.470	0.167	0.032	-0.091	-0.367	-0.238			
841	0.255	0.097	0.058	-0.306	-0.436	-0.212			
842	0.635	0.128	0.024	0.074	-0.405	-0.246	+		
843	0.530	0.102	0.034	-0.031	-0.431	-0.236			
844	0.295	0.059	0.008	-0.266	-0.474	-0.262			
845	0.379	0.160	0.045	-0.182	-0.373	-0.225			
846	0.510	0.117	0.164	-0.051	-0.416	-0.106			
847	0.496	0.063	0.016	-0.065	-0.470	-0.254			
848	0.392	0.157	0.121	-0.169	-0.376	-0.149			
849	0.234	0.299	0.071	-0.327	-0.234	-0.199			
850	0.395	0.138	0.106	-0.166	-0.395	-0.164			
851	0.435	0.095	0.074	-0.126	-0.438	-0.196			
852	0.364	0.387	0.026	-0.197	-0.146	-0.244			
853	0.268	0.061	0.034	-0.293	-0.472	-0.236			
854	0.440	0.112	0.034	-0.121	-0.421	-0.236			
855	0.424	0.135	0.037	-0.137	-0.398	-0.233			
856	0.529	0.205	0.066	-0.032	-0.328	-0.204			
857	0.502	0.297	0.124	-0.059	-0.236	-0.146			
858	0.333	0.208	0.021	-0.228	-0.325	-0.249			
859	0.658	0.235	0.045	0.097	-0.298	-0.225	+		
860	0.259	0.085	0.166	-0.302	-0.448	-0.104			
861	0.398	0.073	0.018	-0.163	-0.460	-0.252			
862	0.341	0.082	0.174	-0.220	-0.451	-0.096			
863	0.489	0.079	0.045	-0.072	-0.454	-0.225			
864	0.466	0.046	0.095	-0.095	-0.487	-0.175			
865	0.427	0.057	0.034	-0.134	-0.476	-0.236			
866	0.213	0.049	0.040	-0.348	-0.484	-0.230			
867	0.192	0.095	0.069	-0.369	-0.438	-0.201			
868	0.488	0.072	0.116	-0.073	-0.461	-0.154			
869	0.431	0.256	0.035	-0.130	-0.277	-0.235			
870	0.426	0.039	0.055	-0.135	-0.494	-0.215			
871	0.346	0.070	0.069	-0.215	-0.463	-0.201			
872	0.767	0.369	0.139	0.206	-0.164	-0.131	+		
873	0.126	0.331	0.159	-0.435	-0.202	-0.111			
874	0.394	0.358	0.085	-0.167	-0.175	-0.185			
875	0.721	0.542	0.184	0.160	0.009	-0.086	+	+	
876	0.642	0.301	0.166	0.081	-0.232	-0.104	+		
877	0.363	0.206	0.205	-0.198	-0.327	-0.065			
878	0.286	0.344	0.147	-0.275	-0.189	-0.123			
879	0.054	0.195	0.127	-0.507	-0.338	-0.143			
880	0.391	0.206	0.122	-0.170	-0.327	-0.148			
881	0.298	0.175	0.166	-0.263	-0.358	-0.104			
882	0.507	0.214	0.215	-0.054	-0.319	-0.055			
883	0.589	0.185	0.196	0.028	-0.348	-0.074	+		
884	0.184	0.366	0.258	-0.377	-0.167	-0.012			
885	0.164	0.116	0.056	-0.397	-0.417	-0.214			
886	0.115	0.197	0.088	-0.446	-0.336	-0.182			
887	0.119	0.176	0.176	-0.442	-0.357	-0.094			
888	0.377	0.232	0.256	-0.184	-0.301	-0.015			
889	0.563	0.188	0.226	0.002	-0.345	-0.044	+		
890	0.371	0.174	0.136	-0.191	-0.359	-0.134			
891	0.718	0.261	0.234	0.157	-0.272	-0.036	+		
892	0.521	0.304	0.166	-0.040	-0.229	-0.104			
893	0.224	0.159	0.110	-0.337	-0.374	-0.161			
894	0.317	0.174	0.151	-0.244	-0.359	-0.119			
895	0.254	0.109	0.133	-0.307	-0.424	-0.137			
896	0.247	0.140	0.028	-0.314	-0.393	-0.242			
897	0.096	0.146	0.150	-0.465	-0.387	-0.120			
898	0.282	0.214	0.154	-0.279	-0.319	-0.116			

Serum	IgG(1.0)	IgA(0.5)	IgE(0.5)	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
899	0.190	0.104	0.156	-0.371	-0.429	-0.114			
900	0.220	0.190	0.047	-0.341	-0.343	-0.223			
902	0.401	0.254	0.125	-0.160	-0.279	-0.145			
903	0.403	0.183	0.250	-0.158	-0.350	-0.020			
905	0.133	0.059	0.112	-0.428	-0.474	-0.158			
904	0.244	0.426	0.102	-0.317	-0.107	-0.168			
906	0.166	0.148	0.022	-0.395	-0.385	-0.248			
907	0.302	0.081	0.166	-0.259	-0.452	-0.104			
908	0.439	0.125	0.160	-0.122	-0.408	-0.110			
909	0.237	0.052	0.208	-0.324	-0.481	-0.062			
910	0.253	0.183	0.134	-0.308	-0.350	-0.136			
911	0.245	0.108	0.096	-0.316	-0.425	-0.174			
912	0.500	0.375	0.166	-0.061	-0.158	-0.104			
913	0.380	0.212	0.112	-0.181	-0.321	-0.158			
914	0.472	0.551	0.026	-0.089	0.018	-0.244		+	
915	0.424	0.164	0.099	-0.137	-0.369	-0.171			
917	0.191	0.270	0.035	-0.370	-0.263	-0.235			
918	0.323	0.201	0.272	-0.238	-0.332	0.002			+
919	0.082	0.090	0.026	-0.479	-0.443	-0.244			
920	0.159	0.165	0.067	-0.402	-0.368	-0.203			

Serum	IgG	IgA	IgE	IgG-0.561	IgA-0.533	IgE-0.270	IgG+	IgA+	IgE+
40	0.358	0.426	0.051	-0.203	-0.107	-0.219			
41	0.903	1.132	0.190	0.342	0.599	-0.080	+	+	
50	0.187	0.372	0.173	-0.374	-0.161	-0.097			
51	0.379	0.374	0.227	-0.182	-0.159	-0.043			
52	0.441	0.322	0.216	-0.120	-0.211	-0.054			
53	0.512	0.523	0.242	-0.049	-0.010	-0.028			
54	0.811	1.096	0.237	0.250	0.563	-0.033	+	+	
93	1.122	0.313	0.211	0.561	-0.220	-0.059	+		
94	0.297	1.339	0.253	-0.264	0.806	-0.017		+	
123	0.445	0.227	0.197	-0.116	-0.306	-0.073			
124	0.979	0.622	0.264	0.418	0.089	-0.006	+	+	
125	0.453	0.502	0.135	-0.108	-0.031	-0.135			
188	0.530	0.297	0.243	-0.031	-0.236	-0.027			
190	0.682	0.676	0.156	0.121	0.143	-0.114	+	+	
193	0.495	0.338	0.135	-0.066	-0.195	-0.135			
195	0.253	0.211	0.073	-0.308	-0.322	-0.197			
213	0.407	0.291	0.164	-0.154	-0.242	-0.106			
223	0.205	0.288	0.190	-0.356	-0.245	-0.080			
236	0.551	0.752	0.224	-0.010	0.219	-0.046		+	
237	0.369	0.234	0.234	-0.192	-0.299	-0.036			
290	0.938	0.306	0.250	0.377	-0.227	-0.020	+		
303	0.458	0.280	0.225	-0.103	-0.253	-0.045			
304	0.568	0.266	0.272	0.007	-0.267	0.002	+		+
306	0.508	0.419	0.156	-0.053	-0.114	-0.114			
308	0.310	0.507	0.216	-0.251	-0.026	-0.054			
312	0.585	0.225	0.273	0.024	-0.308	0.003	+		+
313	0.268	0.340	0.161	-0.293	-0.193	-0.109			
314	0.260	0.505	0.164	-0.301	-0.028	-0.106			
320	0.462	1.012	0.244	-0.099	0.479	-0.026		+	
323	0.411	0.580	0.160	-0.150	0.047	-0.110		+	
329	0.521	0.282	0.172	-0.040	-0.251	-0.098			
441	0.330	0.253	0.218	-0.231	-0.280	-0.052			
444	0.358	0.305	0.064	-0.203	-0.228	-0.206			
446	0.444	0.299	0.162	-0.117	-0.234	-0.108			
452	0.533	0.428	0.157	-0.028	-0.105	-0.113			
476	0.543	0.267	0.165	-0.018	-0.266	-0.105			
478	0.420	0.263	0.167	-0.141	-0.270	-0.103			
480	0.433	0.205	0.165	-0.128	-0.328	-0.105			
481	0.428	0.719	0.122	-0.133	0.186	-0.148		+	
482	0.489	0.318	0.190	-0.072	-0.215	-0.080			
484	0.435	0.248	0.076	-0.126	-0.285	-0.194			
487	0.626	0.869	0.105	0.065	0.336	-0.165	+	+	
488	0.672	1.202	0.031	0.111	0.669	-0.239	+	+	
489	0.675	0.693	0.195	0.114	0.160	-0.075	+	+	
492	0.349	0.283	0.147	-0.212	-0.250	-0.123			
498	0.253	0.217	0.110	-0.308	-0.316	-0.160			
500	0.378	0.281	0.049	-0.183	-0.252	-0.221			
505	0.156	0.290	0.182	-0.405	-0.243	-0.088			
506	0.194	0.280	0.175	-0.367	-0.253	-0.095			
507	0.235	0.396	0.215	-0.326	-0.137	-0.055			
508	0.141	0.325	0.165	-0.420	-0.208	-0.105			
510	0.840	0.877	0.149	0.279	0.344	-0.121	+	+	
517	0.736	0.602	0.192	0.175	0.069	-0.078	+	+	
518	0.231	0.355	0.164	-0.330	-0.178	-0.106			
519	0.238	0.242	0.177	-0.323	-0.291	-0.093			
521	0.196	0.248	0.053	-0.365	-0.285	-0.217			
526	0.343	0.650	0.197	-0.218	0.117	-0.073		+	
614	0.329	0.383	0.135	-0.232	-0.150	-0.135			
615	0.117	0.365	0.174	-0.444	-0.168	-0.096			
628	0.447	0.597	0.179	-0.114	0.064	-0.091		+	
631	0.198	0.571	0.154	-0.363	0.038	-0.116		+	
635	0.488	0.280	0.064	-0.073	-0.253	-0.206			
637	0.435	0.191	0.124	-0.126	-0.342	-0.146			
640	0.670	0.385	0.266	0.109	-0.148	-0.004	+		
644	0.108	0.303	0.177	-0.453	-0.230	-0.093			
676	0.599	0.505	0.120	0.038	-0.028	-0.150	+		
678	0.299	0.341	0.227	-0.262	-0.192	-0.043			
679	0.574	0.981	0.143	0.013	0.448	-0.127	+	+	
682	0.643	0.310	0.251	0.082	-0.223	-0.019	+		
684	0.213	0.331	0.144	-0.348	-0.202	-0.126			
774	0.550	0.701	0.123	-0.011	0.168	-0.147		+	
779	0.666	0.503	0.215	0.105	-0.030	-0.055	+		
842	0.295	0.268	0.015	-0.266	-0.265	-0.255			